Impact of Innate and Adaptive Immunity on Epstein-Barr Virus Infection and Persistence

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We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium a benefit for humanity.

Marie Curie

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To my parents

1. Summary

The human B-lymphotropic Epstein-Barr virus infects over 90% of the human adult population. Following primary infection, EBV establishes a latent infection and life-long persistence within the memory B cell pool. Eventually, latent infection may lead to the formation of several EBV-associated malignancies such as Burkitt's lymphoma, Hodgkin's disease or post-transplant lymphoproliferative disease. An important cofactor influencing the oncogenic transformation of EBV-infected B cells is chronic immune activation as observed in HIV or chronic malaria infection in areas of sub-Saharan Africa.

Models of how EBV establishes persistence in B cells hold that EBV initially enters the nasopharynx-associated lymphatic tissue including tonsils to infect either a naïve or a germinal center B cell. Expression of EBV latent proteins is thought to drive antigen-independent differentiation to memory B cells, which exit the lymphatic tissue to circulate within the body. Upon homing back to the tonsil and contact with antigen terminal differentiation of the memory B cell to plasma cells is induced and EBV reactivates from latency.

As a functional, antigen-selected B cell receptor is a prerequisite for efficient activation of memory B cells, we tackeled the question whether EBV is also able to directly infect memory B cells that successfully underwent affinity maturation by antigen. Indeed, we show that memory B cells from tonsils are susceptible to EBV infection, thereby providing an alternative route for EBV to establish persistence within the host. The ability of EBV to infect memory B cells is regulated via the expression of β_1 integrin, a recently identified coreceptor for EBV specifically expressed on NALT-originating memory B cells and by the follicular dendritic cell network of the germinal centers, which we could identify as a reservoir for EBV transferring infectious virus preferentially to memory B cells.

Immune activation, as measured by the expression levels of proinflammatory cytokines interleukin 12, interferon γ or interleukin 2 could be demonstrated to influence the balance between EBV latent and lytic infection, favoring latent infection of B cells. Especially Toll-like receptor 9, which is chronically stimulated during malaria infection leads to an inhibition of EBV replication, thereby leading to an increased number of latently infected cells which then in turn increase the likelihood of an oncogenic transformation.

2. Zusammenfassung

Das B-lymphotropische Epstein-Barr Virus findet sich bei über 90% der humanen Weltbevölkerung. Nach Primärinfektion etabliert EBV eine lebenslang ausdauernde latente Infektion innerhalb der Gedächtnis B-Zellen des Wirtes. Eventuell kann diese latente Infektion zu mit EBV assoziierten Tumoren wie dem endemischen Burkitt's Lymphom, der Hodgkin Krankheit oder Lymphomen nach Organtransplantationen führen. Ein wichtiger Kofaktor, für die onkogene Transformation der EBV-infizierten B-Zellen ist eine chronische Aktivierung des Immunsystems, wie bei einer HIV- oder Malariainfektion zu beobachtet ist.

Modelle für die Etablierung der latenten Infektion von EBV gehen davon aus, dass EBV in den Tonsillen entweder naïve oder Keimzell-B-Zellen infiziert. Über virale Proteine kommt es dann zu einer Antigen-unabhängigen Reifung zu Gedächtnis B-Zellen. Nach der Rückkehr dieser EBV-infizierten Gedächtnis-B-Zellen und Kontakt mit fremdem Antigen, wird EBV während der endgültigen Reifung zu Plasmazellen reaktiviert.

Da für eine Effiziente Aktivierung von Gedächtnis-B-Zellen eine Antigen-Selektion unumgänglich ist, muss auch eine direkte Infektion von Gedächtnis-B-Zellen mit EBV möglich sein. Tatsächlich zeigten *in vitro*-Untersuchungen, dass Gedächtnis-B-Zellen aus dem Nasopharynx mit EBV infizierbar sind. Diese sind bereits durch Antigenkontakt selektiert worden und können somit auch zu Plasmazellen reifen. Die Infektion von Gedächtnis-B-Zellen wird vor allem durch zwei Faktoren gewährleistet. β_1 Integrine welche spezifisch auf Gedächtnis-B-Zellen exprimiert werden, die in den Tonsillen gebildet wurden werden von EBV für die Bindung an die Zelle benutzt. Hierbei wird ein Signalübertragungsweg ausgelöst, der über eine erhöhte Depolymerisation des Aktin-Cytoskeletts zu einer erhöhten Aufnahme von EBV in die Zelle führt. Des Weiteren kommt es in den Keimzentren der Tonsille zu einem Transfer von EBV von follikulären Dendritischen Zellen auf Gedächtnis-B-Zellen, was ebenfalls zu einer effizienteren Infektion führt.

Eine erhöhte Grundaktivierung des Immunsystems, hauptsächlich über Interleukin 12, Interferon γ und Interleukin 2 bewirkt eine Inhibierung der Replikation von EBV und somit zu einer Anhäufung latent mit EBV infizierten B-Zellen. Besonders ausgeprägt ist dieser Effekt bei chronischer Aktivierung von Toll-like Rezeptor 9 zu beobachten, der auch von Malaria stimuliert wird. Durch die erhöhte Anzahl latent infizierter Zellen ist auch die Wahrscheinlichkeit erhöht, dass es zu einer onkogenen Mutation der Zellen kommt.

3. Introduction

3.1. Epstein-Barr virus

The Epstein-Barr virus (EBV), a herpesvirus of the γ-L or lymphocryptovirus (LCV) genus was discovered through the observations of Denis Burkitt and Anthony Epstein of multifocal jaw tumors in children in Kampala in 1964[1]. The tumors were lymphomas prevalent in children in climates supportive of holoendemic malaria[2-4]. After successful culturing of the lymphoma cells, they could identify a herpesvirus in electron micrographs[1]. The Burkitt's lymphoma (BL) tumor virus differed from known human herpesviruses as it was noninfectious for cultured cell lines and nonreactive with antibodies to other human herpesviruses. Besides the Kaposi's sarcoma-associated herpesvirus (KSHV), EBV is the only known human γ-herpesvirus.

EBV displays the two classical characteristics of the γ-herpesvirus family (Table 1). Firstly, it is widespread in all human populations, with more than 90% of adults positive for serum IgG antibodies. Second, the virus persists for life in the immune host and, can be rescued *in vitro* from circulating lymphocytes when using spontaneous B-cell transformation.

Primary Infection with EBV is usually asymptomatic in infants and toddlers, whereas infection of older children, adolescents and adults may manifest with the clinical symptoms of infectious mononucleosis, a vigorous immune hyperactivation[5]. These symptoms include fever, enlarged tonsils and lymph nodes, sore throat, muscle weakness, enlarged spleen and abdominal pain[6]. Infectious mononucleosis is generally self-limiting and only symptomatic and/or supportive treatments are used. During primary infection, EBV establishes a life-long persistence within the B cell pool of the host.

Table 1. Human herpesviridae

Designation	Vernacular name (Synonyms)	Subfamily and Genus
Human HHV1	Herpes simplex virus 1	αS
Human HHV2	Herpes simplex virus 2	αS
Human HHV3	Varizella-Zoster virus	αS
Human HHV4	Epstein-Barr virus	γL
Human HHV5	Cytomegalovirus	βC
Human HHV6A	HHV-6 variant A	βR
Human HHV6B	HHV-6 variant B	βR
Human HHV7		βR
Human HHV8	Kaposi's sarcoma-associated HV	βR

3.2. EBV-associated malignancies

EBV is associated with various lymphomas and cancers. These include immunoblastic lymphoma of immunosuppressed, Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), post-transplant lymphoproliferative disease arising in organ transplant patients as well as several nasal T/NK lymphoma, lymphoepitheliomas of the nasopharynx, thymus and stomach and leiomyosarcomas[7,8].

Table 2. EBV-associated malignancies

Malignancy	EBV-association (%)	Incidence rates per 100 000	Other infections	Host immune status	Number of expressed EBV genes
Burkitt's lymphoma					
endemic	>95	6-15	malaria	competent	
sporadic	15-88	0.13	-	competent	
AIDS-related	30-40	608*	HIV	compromised	
Hodgkin's disease	40-50	2-4	-	competent	
AIDS-associated lymphoma					
Immunoblastic	>95	1534*	HIV	compromised	
Primary CNS	70	572*	HIV	compromised	
Post-transplant lymphoma	>95	n.a.	HIV	compromised	

* US patients with AIDS

As in persistent infection for different B cell differentiation stages, the array of EBV genes that is expressed in EBV-associated malignancies differs[9]. The more immune competent the host, the less genes EBV expresses to evade immune recognition.

Table 3. EBV gene expression in EBV-associated malignancies

Gene Expression program	EBNA1	EBNA2-6 EBNA-LP	LMP1 LMP2A LMP2B	EBER1 EBER2	Malignancy
Latency I	+	-	-	+	Burkitt's lymphoma Gastric carcinoma
Latency II	+	-	+	+	Nasopharyngeal carcinoma Hodgkin's disease Nasal NK/T lymphoma
Latency III	+	+	+	+	AIDS-associated lymphoma PTLD
Other	+	EBNA2 only	-	+	Leiomyosarcoma

3.2.1. *Burkitt's lymphoma*

Burkitt lymphoma (BL) is an aggressive B-cell malignancy, which can be classified into three forms which differ in geographic distribution, Epstein–Barr virus (EBV) association, and immunocompetence status: endemic (eBL), sporadic (sBL) and HIV-associated BL[4,8,10,11]. There is a low background incidence of BL worldwide (sBL), which is rarely associated with EBV and accounts for 1–2% of adult lymphoma in Western Europe and America. In contrast eBL is associated with (EBV) in over 95% of cases and is predominant in the equatorial belt of Africa and other parts of the world where malaria is hyperendemic. BLs that display an intermediate association with EBV have also been documented in Egypt and Brazil, where up to 87% of tumors are EBV positive and BL occurs in HIV carriers, where tumors can develop prior to the severe immunosuppression coincident with the onset of AIDS. Approximately 30% of such AIDS-associated tumors are EBV-positive. Endemic EBV-associated BL has an incidence of 5–10/100 000 children and accounts for up to 74% of childhood malignancies in the African equatorial belt. In contrast to sBL, which most frequently involves tumors of the abdomen, eBL often presents in the jaw or kidneys but may also occur in the abdomen, ovaries, facial bones and other extranodal sites[12]. The cancer has one of the highest cell proliferation rates of any human tumor (doubling time of tumor 24–48 h).

A hallmark of BL is the translocation of the proto-oncogene *MYC* into one of the immunoglobulin loci, resulting in constitutive activity of this transcription factor[10]. This misregulated expression of *MYC* in turn is responsible for the rapid proliferation and tumor formation. The structure of the chromosome breakpoints of the *MYC* translocation strongly indicates that the translocations occurred either as a mistake of class-switch recombination or somatic hypermutation (SHM)[13]. Moreover, mutations of the gene encoding the tumor suppressor p53 are found in a third of cases of BL, and there is also evidence for genetic alterations of the putative tumor suppressor gene retinoblastoma-like 2 (RB2) in most cases of eBL and sBL. Additionally, EBV drives proliferation of the cell by initiating the latency III growth program before shutting-down its gene expression and establishment of the latent infected B cell pool. In BL, key steps of malignant transformation probably occur in an EBV-infected B cell residing in a germinal center[14,15].

Histologically, BL cells are monomorphic medium sized cells with round nuclei, a number of nucleoli and abundant cytoplasm[12]. Tumors contain high numbers of macrophages, which phagocytose apoptotic debris. BL tumor cells usually express IgM, B-cell markers such as CD19, CD20 and CD22 and markers of GC centroblasts such as CD10, *BCL6* and the

human GC-associated lymphoma (HGAL) protein. The cell surface phenotype of BL tumor cells reflects a GC origin but the site of tumor growth is frequently the jaw or ovary, neither of which normally contains GCs. However, the tumor cells have undergone SHM, a feature of the GC reaction during B-cell activation and differentiation. Moreover, the breakpoint in the Ig gene to which *MYC* is transferred in eBL occurs at the V(D)J region, suggesting that translocation occurs during V(D)J recombination. The J segments flanking *MYC* translocated breakpoints typically exhibit deletions and/or additions of base pairs characteristic of normal Ig V(D)J segment rearrangement. This is a process catalysed by B-cell specific V(D)J recombinase activating enzymes RAG-1/2 which are expressed in both pre-B cells and GC B cells[16]. In contrast, the chromosomal breakpoint in sBL and HIV-associated BL occurs most commonly in the class switch region, but since both somatic hypermutation and class switching are events that are normally confined to GC B cells and GC centroblast markers are expressed on BL cells, the BL progenitor cells most likely arise from B cells subjected to chromosomal rearrangements in the GC.

There is some evidence that the cell of origin may be a post-GC or memory B cell re-entering the GC and may differ in EBV-positive and negative tumors, but whichever is the cell of origin, it is clear that GC involvement is critical to the pathogenesis of this disease both in terms of *MYC* translocation events and the contribution of co-factors such as EBV, malaria or HIV infection.

EBNA1 is normally the only EBV-encoded protein expressed by BL cells. Its oncogenic potential is debated. EBERs, which are also transcribed, can induce the expression of interleukin-10 (IL-10) and mediate resistance to interferon-α (IFN-α), which might support tumor growth and survival.

3.2.2. *Hodgkin's disease*

Hodgkin lymphoma is characterized by atypical, large tumor cells known as Hodgkin and Reed–Sternberg (HRS) cells[17-20]. These cells usually represent less than 1% of cells in the tumor tissue. Most cells in the tumor are non-malignant T cells, B cells, eosinophils and others. Based on differences in the histology and immunophenotype of HRS cells, a classical form of Hodgkin lymphoma, accounting for 95% of cases, is distinguished from a lymphocyte predominant form. HRS cells of lymphocyte-predominant Hodgkin lymphoma are always EBV negative, whereas in ~40% of cases of classical Hodgkin lymphoma in the Western world, EBV is detected in the lymphoma cells. In EBV-positive cases, three EBV proteins are expressed, that is EBNA1, LMP1 and LMP2A (latency II). The cellular origin of HRS cells of

classical Hodgkin lymphoma has long been unclear, partly because they do not resemble any normal haematopoietic cell type. Single-cell molecular analysis showed that HRS cells in nearly all cases derive from B cells[15]. The rearranged immunoglobulin V genes of HRS are somatically mutated, but lack intraclonal V gene diversity, indicating that the SHM machinery is silenced in the tumor cells. Germinal-centre B cells that acquire such 'crippling' mutations are normally efficiently eliminated by apoptosis in the germinal centre. The expression pattern of EBV-encoded genes in HRS cells indicates a role for EBV in the rescue and transformation of pre-apoptotic GC B cells[21]. Two of the main survival signals for GC B cells are mediated through the BCR and through stimulation of CD40. These signals can presumably be replaced by LMP2A and LMP1, respectively, both of which are expressed by EBV-positive HRS cells.

HRS cells have lost the expression of most B cell-specific genes crucial for BCR signaling. Perhaps, the global loss of the B cell identity that is associated with the generation of HRS cells allows these cells to escape the normal selection forces of B-lineage cells to express a functional antigen receptor and allows survival of the transformed cells in the absence of a BCR.

3.2.3. *Post-transplant lymphoma*

The T-cell immunosuppressive therapy given to patients after transplantation is associated with a greatly increased risk of lymphoproliferative diseases. These post-transplantation lymphoproliferative diseases (PTLDs) are nearly always of B cell origin and EBV positive[22,23]. Most probably, the suppression of T-cell responses allows uncontrolled proliferation of EBV-transformed B cells. PTLDs can present as polyclonal expansions, but many cases show oligoclonality or monoclonality. In the latter situation, some EBV-transformed B cells might have acquired additional transforming events, finally leading to the outgrowth of a malignant B cell clone from an initially poly- or oligoclonal expansion. The EBV-positive B cells in PTLDs often have a latency III profile, but more restricted gene-expression patterns are also observed. The detection of SHM activity and/or crippling mutations in a considerable fraction of monoclonal PTLD clones indicates that these lymphomas often derive from germinal-center B cells.

3.3. Latency programs of EBV at and after primary infection

Epstein-Barr virus infection of primary B cells, *in vitro*, results in the cells becoming latently infected. 3% to 10% of those cells start proliferating as immortal or transformed

lymphoblastoid cell lines (LCL)[24]. The mechanism of this remarkable effect depends on the expression of nine viral latent proteins that are under the control of a master regulatory transcription factor, EBV nuclear antigen 2 (EBNA2)[25]. *In vitro* events are very different from what occurs in the blood of healthy carriers of the virus. Here, up to 1 in 10^6 B cells are also latently infected with EBV. These cells are all memory B cells that are in a resting state and they express no viral proteins[26]. They are recognizable as EBV infected by their expression of EBERs (non-coding EBV RNAs). Cells that express the growth program are found only in the lymph nodes and possess a naïve phenotype. Depending on the differentiation stage of the infected B cell, EBV activates a distinct gene expression program (Table 2)[26]. The Growth program is defined as the EBV expression pattern detected when EBV infects naïve B-cells (EBNA1-6 (EBV nuclear antigen), LMP1 (latent membrane protein 1), LMP2A, LMP2B, and EBERs (EBV-encoded RNA)). In EBV-infected germinal center (GC) B cells, the Default program is predominantly switched on, comprised by the expression of EBNA1, LMP1, LMP2A, LMP2B, and EBERs. In resting memory B cells carrying EBV, viral gene expression is tightly restricted to the expression of EBERs with EBNA1 and LMP2 only being expressed during cell division.

Besides the different latency programs, EBV also reactivates from latency from time to time, initially activated by expression of BZLF1 and followed by transcription of all lytic genes, virus assembly and budding.

Table 4. Patterns of EBV gene expression depending on B cell differentiation stage

Gene Expression program	EBNA1	EBNA2-6 EBNA-LP	LMP1 LMP2A LMP2B	EBER1 EBER2	Site of Infection
Latency 0	-	-	-	+	Resting memory B cells
Latency I	+	-	LMP2 only	+	Dividing memory B cells
Latency II (Default program)	+	-	+	+	Germinal center B cells
Latency III (Growth program)	+	+	+	+	Naive B cells

3.4. EBV entry

Eight virus glycoproteins have been implicated in some way in EBV entry into either a B cell or an epithelial cell. EBV requires at least two host cell factors for efficient binding and entry into B cells. CD21 is an efficient initial EBV receptor and is also the receptor for the C3d component of complement[27-31]. Purified CD21 binds to EBV and can block B-cell infection[32-34]. The most abundant EBV outer envelope glycoprotein, gp350/220 is binding to EBV thereby facilitating virus adsorption to the cell[35,36]. Furthermore, soluble gp350/220 can saturate B-lymphocyte receptors and block virus infection, confirming a key role for gp350/220 in virus adsorption[37]. Binding of gp350/220 also triggers capping of CR2 and endocytosis of the virus[38]. CR2 can function as a signal transducer both independently and as part of a signal transduction complex that includes CD19 and CD35[39]. Cross-linking by gp350/220 activates NF-κB[40] and induces interleukin-6 via a protein kinase C pathway[40]. None of these signaling events may be critical to penetration of the cell membrane, but they clearly have potential consequences for downstream events in infection.

Fusion of the EBV envelope with both a B cell and an epithelial cell membrane requires three glycoproteins, gH/gL and gB, which are conserved throughout the herpesvirus family and which have been referred to as the core fusion machinery[41,42]. Within the three-part complex of gHgLgp42 in the virus, this interaction is now thought to provide the trigger for B-cell fusion. Binding of gp42 to HLA class II shows some allelic specificity in that nonfunctional HLA-DQ alleles have been identified[43], but since all three alleles, HLA-DP, HLA-DQ, and HLA-DR, can be used, it seems unlikely that this has a major impact in an outbred human population.

Recently, we and others have reported that EBV might use another glycoprotein, BMRF2 as an attachment factor to epithelial cells and memory B cells from the tonsil[44-46]. By binding to its cellular receptor, the β_1 subfamily of integrins, EBV triggers actin depolymerization through activation of focal adhesion kinase (FAK), *c*-Src and PI_3 kinases, thereby enhancing susceptability of these cells to EBV infection[47-49].

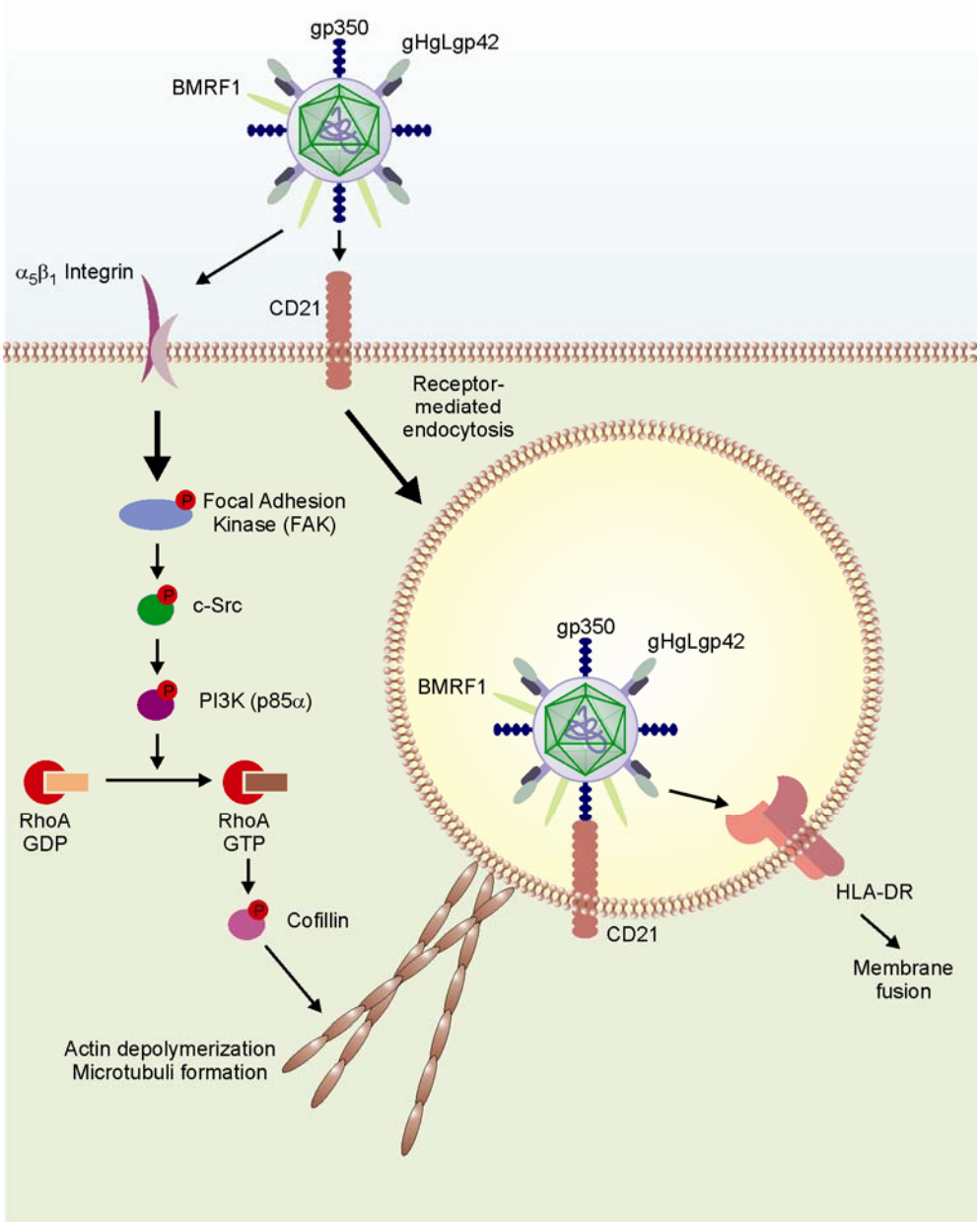

Figure 1. Molecular events required for efficient infection of B cells with EBV. After initial binding of EBV gp350 to cellular CD21, receptor-mediated endocytosis is triggered. Within the endosomes, a viral glycoprotein complex consisting of gH, gL and gp42 binds to HLA class II molecules ultimately leading to membrane fusion of EBV with the endosomal membrane. A fifth EBV glycoprotein, BMRF2, may also bind to cellular β_1 integrins, thereby mediating actin depolymerization followed by more efficient EBV entry and nuclear transport.

3.5. Establishment of Epstein-Barr virus persistence

3.5.1. B cell maturation

B cell development occurs in a series of discrete stages beginning within the fetal liver as early as eight weeks gestation, and maintenance in bone marrow for the rest of the life[50,51]. The first recognizable developmental stage is the rapidly-dividing pre-B cell which

synthesizes cytoplasmic IgM but lacks surface receptors for antigen[52,53]. Immature B lymphocytes emerging from pre-B cells express surface IgM, but are uniquely susceptible to inactivation as a consequence of specific antigen binding. After finishing the development to a mature B cell, it exits the bone marrow and homes to the secondary lymphoid organs[54], where all further differentiation steps take place. Dendritic cells, which collect antigen in the periphery and at the epithelial barriers, present them to the antigen-naïve B cells and T cells and initiate rapid proliferation of the responsive B cell clones which then form the germinal center (GC). It is here, where the antigen-driven selection takes place, leading to the generation of highly antigen-specific memory B cells. Furthermore, the dendritic cells with bound antigen serve to stimulate T_H2 differentiation of $CD4^+$ T cells which is an important cofactor for the GC reaction. Only if the B cell recognizes the antigen with its BCR, it forms a so-called immunological synapse with the DC and the T cell, receiving survival signals through the BCR and a CD40-CD40L interaction with the T cell. This in turn activates the somatic hypermutation machinery in the B cell which ultimately serves to increase the antigen specificity of the BCR by inserting mutations within distinct hotspot regions within the immunoglobulin variable region. In the course of the selection process, the T cells secrete a defined pattern of cytokines and chemokines which then initiate the last step of antigen-driven selection, the isotype switch. This leads to the excision of immunoglobulin constant regions, leading to the expression of IgG, IgA, or IgE instead of IgM. The pattern of secreted cytokines regulates, which immunoglobulin isotype is produced. Following successful completion of the GC reaction, the B cell exits the lymphoid tissue as a memory B cell to patrol the periphery for cognate antigen. Engagement of the BCR by an apt antigen then initiates the last step in B cell differentiation, terminal differentiation to an antibody-producing plasma cell synthesizing IgM, IgD, IgG, IgA, or IgE immunoglobulins, respectively.

3.5.2. Establishment of EBV persistence

In healthy, EBV-seropositive individuals, EBV persistence in the peripheral blood is strictly confined to $CD27^+$ IgD^- memory B cells and absent from the IgD^-, $CD27^-$, naïve B cell subset[55,56]. This has given rise to a model for EBV colonization of the B cell pool in which virus infection of a naïve B cell mimics the process of antigen-driven differentiation and drives that cell's progeny to acquire memory status[57]. The generated “phenotypic” memory B cells that bypassed affinity maturation then exit the tonsil and enter the peripheral circulation. Upon activation of these EBV-harboring cells homing back to the tonsils by so far unknown

mechanisms, terminal differentiation to plasma cells is initiated by the unfolded protein pathway ultimately leading to X-Box binding protein (XBP)-1 expression[58]. This in turn activates EBV lytic replication and the production of progeny virions.

Even though this model has been widely accepted, additional findings suggest in addition EBV infection occuring at different stages later than naïve B cells.

3.5.2.1. Primary infection of naïve B cells

David Thorley-Lawson was the first to postulate a model for EBV persistence based on an initial infection of naïve B cells within the tonsil[56,57,59-66]. This is based on the finding of only naïve B cells within the tonsil expressing the growth program required for the transformation of B cells *in vitro*[61]. The model holds that, upon infection of a naïve B cell, EBV activates B cells to become proliferating blasts so that they can then differentiate into resting memory B cells simulating the process of the GC reaction[67]. This process parallels the activation of a naive B cell on exposure to an antigen. It is in the GC that an activated naive B cell blast that is responding to a foreign antigen during an immune response undergoes the transition into a long-lived memory B cell. The antigen-activated B cell is rescued through entry into the pool of memory B cells when it receives signals from antigen and antigen-specific helper T cells. As EBV expresses LMP1 and LMP2 during the default program, thereby mimicking CD40-CD40L interaction and an activated B cell receptor (BCR), it delivers the rescue signals to the latently infected blast[65]. Then the memory cells enter the peripheral circulation. In response to so far unknown signals, memory B cells may differentiate into plasma cells and secrete antibody. If such a cell contains EBV, it will reactivate viral replication and infectious virus will be produced[58,62].

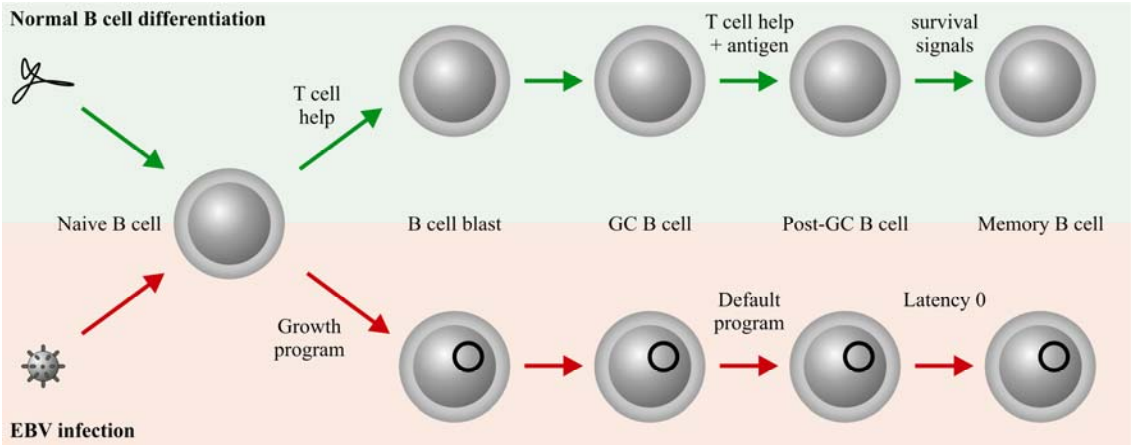

Figure 2. Model for EBV persistence by initial infection of naïve B cells. EBV, not antigen stimulation and T cell help provide the required survival signals necessary for memory B cell differentiation of the EBV-infected naïve B cells (adapted from [57]).

3.5.2.2. Primary infection of germinal center B cells

Even though EBV-infected B cells are only very rarely detectable in the germinal center, it could be shown, that centroblasts and centrocytes are susceptible to EBV infection[19,68-72]. Infected GC B cells express LMP1 and LMP2, providing the cells with survival signals even in the absence of a functional BCR or CD40 signaling[73-76]. This in turn drives the GC B cell to differentiate to a memory B cell, even if the B cell carries a "crippled" BCR, i.e. a BCR unable to respond to foreign antigen[68].

3.5.2.3. Primary infection of memory B cells

Together with the finding that EBV is able to infect GC B cells directly, it was first suggested by us and others that EBV could also directly infect antigen-selected memory B cells[71,77]. Such infection would minimize the risk for the EBV-infected B cell to undergo apoptosis in the GC reaction. Furthermore, it would explain, how EBV-reactivated from infected memory B cells to produce new EB virions. As the memory B cells successfully underwent affinity maturation, somatic hypermutation (SHM) and class-switch recombination (CSR) within the GC prior to EBV infection they will still respond to cognate antigen exposure with high specificity[78,79]. Finally, this model bears another advantage over the previous two models as the memory B cells are able to respond to cognate antigen. Depending on the binding strength of the BCR-antigen-interaction, the cell will either start to proliferate extensively, spreading throughout the host to serve as sentinels for a possible site of pathogen invasion, or the BCR signal will activate the memory B cells which is subsequently undergoing terminal plasma cell differentiation[80]. This in turn activates XBP-1, which activates EBV lytic replication[58,81]. Therefore, the EBV life-cycle can be completed upon terminal differentiation of the EBV-infected memory B cell simply by antigen encounter.

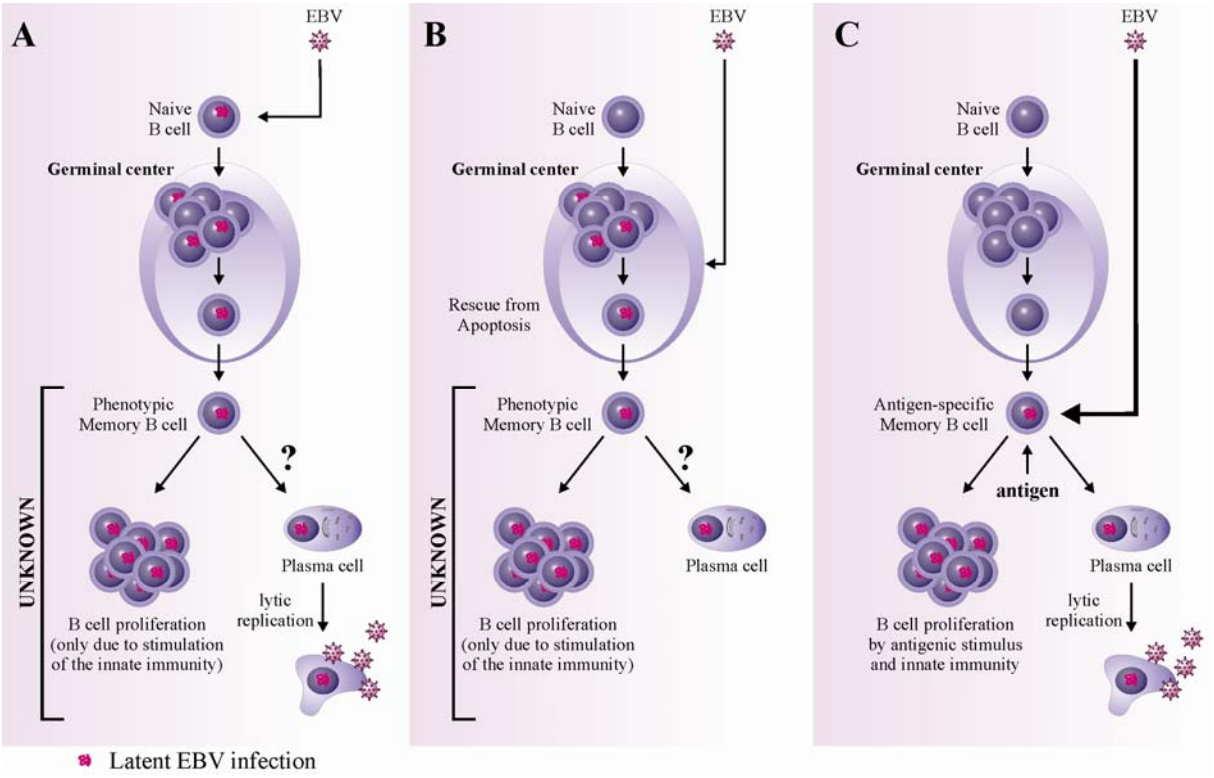

Figure 3. Models for the establishment of EBV persistence. (A) Initial infection of naïve B cells. Naïve B cells are provided with survival signals by EBV gene products instead of antigen-affinity. The resulting memory B cells carry a non-specific BCR not responding to foreign antigen. (B) Initial infection of GC B cells. EBV infects GC B cells currently in the phase of SHM and CSR, resulting in "crippled" BCR-expressing EBV-infected memory B cells. (C) Infection of affinity-matured antigen-specific memory B cells providing the missing explanation how EBV is transmitted due to BCR stimulation and EBV lytic cycle reactivation.

3.5.2.4. The role of follicular dendritic cells in the establishment of EBV persistence

Follicular dendritic cells (FDC) are the body's most potent antigen-presenting cells (APC)[79,82,83]. They present antigens to naive B cells within the GC and are therefore responsible for the positive selection of antigen-specific B cells[82]. Besides presenting antigen to naïve B cells, FDC with bound antigen are also involved in the reselection process of already antigen-specific memory B cells that are frequently homing back to the GC during the secondary immune response[84]. We could show that FDC are directly involved in the infection of memory B cells with EBV[85]. FDC bind EBV via complement receptor 2 (CR2) and presents the virus within the GC. During the positive selection process, EBV is then transferred from the FDC preferentially to memory B cells. The route by which EBV initially enters the GC might be through plasma cells, which were also shown to home to the GC[86].

3.6. B cell homeostasis and EBV

The mucosal immune system can be divided into inductive and effector sites. In inductive sites, antigens sampled from mucosal surfaces stimulate cognate naïve T and B lymphocytes. In effector sites the effector cells perform their action after extravasation and differentiation[54,87-89]. Inductive sites for mucosal immunity consist of organized mucosa-associated lymphoid tissue (MALT) as well as local and regional draining lymph nodes (LNs), whereas the effector sites consist of distinctly different histological compartments, including lamina propria (LP) of various mucosae, stroma of exocrine glands, and surface epithelia. MALT structures resemble LNs with B-cell follicles, intervening T-cell areas, and a variety of antigen-presenting cells (APCs), but they lack afferent lymphatics[87].

Although the gastrointestinal-associated lymphatic tissue (GALT) is the largest and best defined part of MALT, other potentially inductive sites for mucosal B-cell responses are bronchus-associated lymphoid tissue (BALT) and nasopharynx-associated lymphoid tissue (NALT) – in humans particularly the unpaired nasopharyngeal tonsil (often called adenoids) and the paired palatine tonsils[88]. The latter organs make up most of Waldeyer's pharyngeal lymphoid ring and may play a major role for mucosal immunity in human airways. Besides that, NALT is widely accepted to be the portal of entry of EBV. It is in the tonsils, where EBV first encounters and infects B cells.

Previously, we reported that memory B cells are susceptible to EBV infection, if they originate from NALT[46]. Memory B cells generated in other lymphatic organs do not express the required amount of β_1 integrin, therefore leading to inefficient binding and entry of EBV. This implies that EBV, even though it can be employed as a tool to transform memory B cells secreting antibodies against respiratory antigen which led to affinity maturation in the NALT, transformation of memory B cells selected in non-NALT by antigen is not feasible.

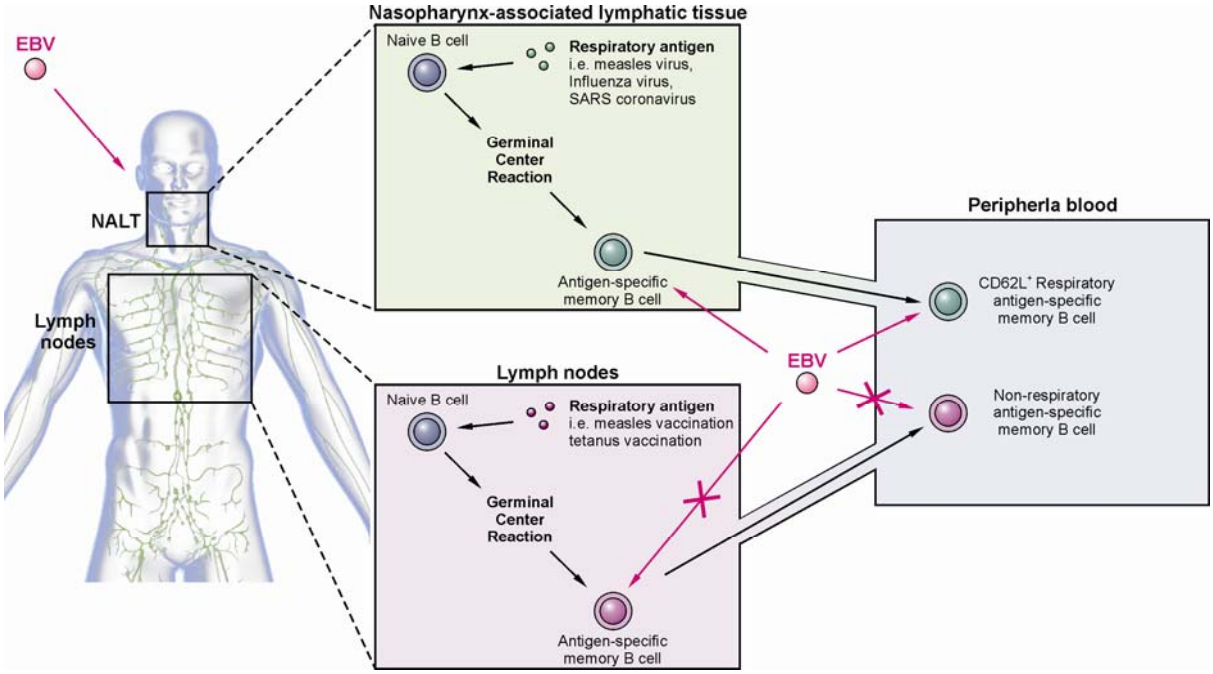

Figure 4. Model of differential susceptibility of memory B cells from diverse lymphatic tissues to EBV infection. EBV enters the body mainly via the saliva and penetrates the oral mucosal epithelium to gain access to the B cell-rich areas of the NALT, including tonsils. Here, EBV is able to directly infect memory B cells that have been generated within the NALT. Other lymphatic organs may eventually also be exposed to EBV, even though memory B cells generated in these organs are less susceptible to EBV infection.

3.7. Cofactors in the genesis of EBV-associated malignancies

Several cofactors have been associated to the formation of EBV-harboring malignancies. The two most important are malaria and HIV.

3.7.1. *Human Immunodeficiency virus (HIV)*

In the setting of a progressing HIV infection accompanied by loss of $CD4^+$ T cells, EBV-harboring brain lymphoma are common and the leading cause for lymphomagenesis in HIV-patients[22]. The theory holds that the lacking immune control through CD4 and $CD8^+$ T cells leads to an unhampered expansion of EBV-infected B cells, driven by the expression of the 'growth program' of EBV.

3.7.2. Plasmodium falciparum *malaria*

The role of malarial infection in the pathogenesis of eBL was supposed for the geographical co-incidence of the two diseases. It is generally thought that the association between malaria and BL arises from a combination of immunosuppression and B-cell activation[3,4,90,91]. For example, cytotoxic T-cell mediated control over the outgrowth EBV-infected B cells is impaired during acute malaria infection, and it has been found that

peripheral EBV loads may be five times higher during acute malaria compared to levels observed during convalescence or in healthy individuals[92]. EBV loads are generally higher in areas of holoendemic malaria compared to areas where malaria is sporadic, and show increased persistence in children with a history of severe rather than mild malaria, possibly owing to higher viral reactivation. Endemic BL also develops at a later age in individuals who have migrated from malaria-free high altitude areas to lower, malaria-endemic areas.

In support of these findings, it has recently been found that the malarial parasite *Plasmodium falciparum* can directly activate B cells via a cysteine-rich interdomain region 1α (C1DR1α) on the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which binds to surface Ig[93]. The activation of B cells by CIDR1α and subsequent protection from apoptosis has been postulated to play a role in enhancing survival of GC B cells bearing oncogenic mutations. In addition to the activation of B cells, it is possible that proliferation of B cells is enhanced by IL-10. Serum levels of this cytokine are raised in children suffering from acute *P. falciparum* malaria compared to healthy controls[2,94]. Protective immunity is only acquired following several years of exposure to the malarial parasite *P. falciparum*, and the intervening immunosuppression in malaria endemic areas may alter the regulation of EBV-positive B cells.

Another recent finding identified that the malaria pigment hemozoin specifically binds DNA and continuously stimulates TLR9[95]. This chronic innate immune stimulation may be one of the key factors governing malignant transformation.

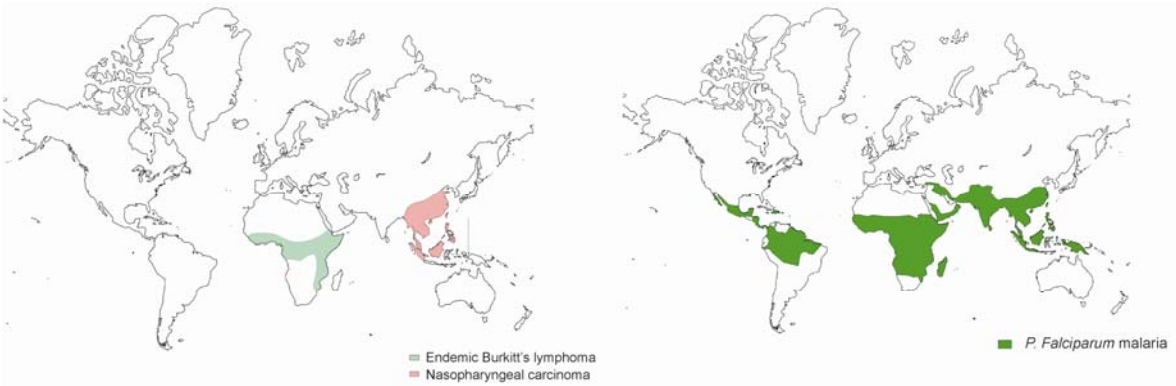

Figure 5. Geographic distribution of EBV-associated malignancies (left) and endemic *P. falciparum* malaria (right).

3.8. Innate immunity and Epstein-Barr virus

Interactions between pathogens and the host they infect are multifactorial. Innate immunity mediates direct antimicrobial effects at the earliest times of primary infections, while slower adaptive and humoral immune responses are being expanded and activated. Innate immunity also delivers important immunoregulatory effects to shape downstream immune responses. Thus, the innate immune system is prepared to act as a first line for sensing infection, to unleash protective defense mechanisms mediated through a high proportion of preexisting cells, and to both direct and support other protective responses.

Toll-like receptors (TLR), the major receptor type of the innate immune system, are a family of transmembrane proteins each of which recognizes specific microbial pathogen-associated molecular patterns (PAMPs) to sense infection and then responds by triggering specific cellular responses[96-99]. Members of the TLR family of molecules, now numbering in excess of ten, bind to various microbial products that can be distinguished as nonself[100].

Besides being the first line of defense through the activation of cytokine production, TLRs were also shown to shape the humoral immune response either by activating somatic hypermutation thereby enhancing the efficiency of the GC reaction or by modulating the process of class-switch recombination favoring the generation of IgG-bearing memory B cells[99].

Most TLR signaling pathways require cellular adapter proteins to start a cascade of phosphorylation-dependent events ultimately culminating in the activation of key transcription factors such as nuclear factor (NF)-κB, JNK or AP-1[96]. These are then responsible for the transcription of an array of proinflammatory cytokines and chemokines. Albeit only six adapter proteins are known to play a role in TLR activation, the response to TLR engagement are multifaceted implying that additional factors besides the type of adapter protein are required to shape TLR signaling[101].

Even though TLRs have initially been linked to antiviral effects, recent data suggest, that several members of the herpesvirus family modify the TLR signaling pathways to maintain their latent infection or evade recognition by other immune cells.

Some EBV viral products have the ability to manipulate the host cellular TLR signaling pathways during latent or lytic phases. Such deregulated signaling by viral products contributes to malignant cell proliferation, survival and viral expansion.

EBV latent membrane protein 1 (LMP1) activates the NF-κB pathway[102-105]. The LMP1 *C*-terminal cytoplasmic tail contains two distinct functional domains, *C*-terminal

activation regions 1 and 2 (CTAR1 and 2). Each CTAR1-TRAF2 and CTAR2-TRADD complex links to the IKK–NIK–NF-κB pathway[106,107]. These unscheduled activations of the NF-κB pathway by viral products are required for virus-induced cellular transformation and cell survival.

Furthermore, LMP1 induces IRF4 expression, an Interferon regulatory factor family member that negatively modulates TLR-mediated IRF-5 activation by competing with IRF-5 for interaction with MyD88[108]. This leads to upregulation of MyD88 and TLR7 followed by increased cellular proliferation.

EBV latent membrane protein 2A (LMP2A) has an *N*-terminal cytoplasmic region containing eight tyrosine residues, which function as the immunoreceptor tyrosine-based activation domain (ITAM) motif[109]. The phosphorylated ITAM region recruits Src-type tyrosine kinases, and this ITAM signalsome-mediated ERK-activation also stimulates the c-Jun/AP-1 pathway[110].

In addition, non-protein products from EBV, EBER1 and 2 are shown to affect host cell signaling. EBER1 and 2 are non-translated viral small RNA abundantly expressed in EBV latently infected cells. EBER activate the NF-κB and IRF3 pathways through association with RIG-I[111,112].

Besides latent EBV gene products, also lytic EBV genes impact the signaling induced downstream of TLRs. ZEBRA, the protein encoded by the immediate-early gene BZLF1 of EBV is on the one hand inducing NF-κB translocation into the nucleus[113], but is also able to inhibit binding of NF-κB to responsive promoters[114].

All of the above-mentioned cellular signaling molecules and transcription factors are also activated downstream of TLR activation. These data show that EBV by itself modulates the innate immune system thereby preventing immune recognition of EBV-infected cells. Furthermore, several pathways of the innate immune system are known to influence the balance between EBV latent and lytic infection. Signaling through TLR9 ultimately leading to the sequestration of IL-12 and IFN-γ downregulates EBV lytic infection through a so far unknown mechanism[115]. Besides that, it could be shown that TLR signaling impacts the EBV-associated growth transformation of B cells[116-120].

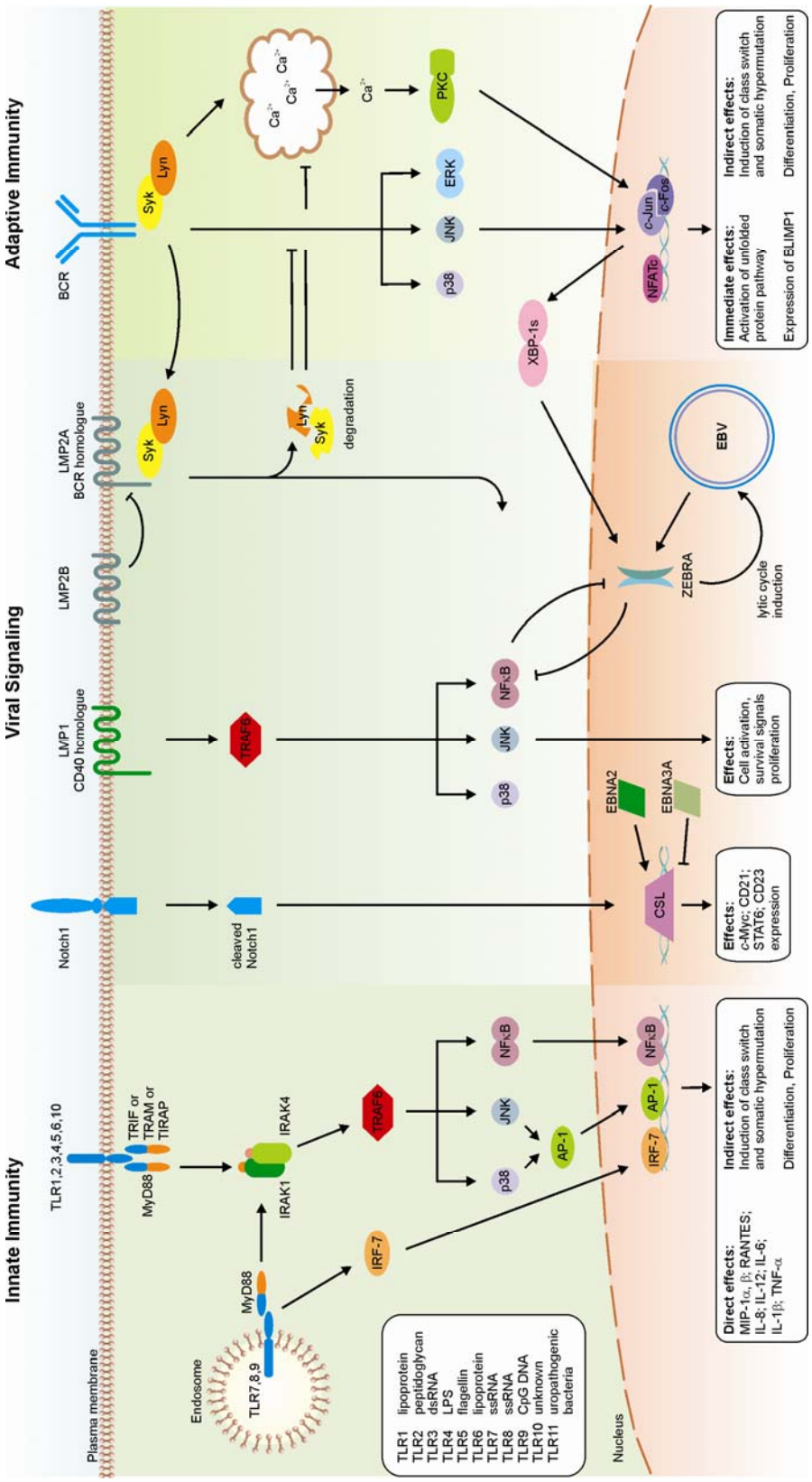

Figure 6. Mutual influence of innate, adaptive immunity and EBV infection in B cells. Signaling pathways regulated downstream of the innate immune receptors of the Toll-like family or the B-cell receptor are also frequently modulated by EBV latency and lytic proteins. Especially the transcription factor NF-κB, which is activated by TLRs could be shown to have a regulatory loop with an EBV immediate-early protein ZEBRA.

4. Subject of investigation

Due to the fact that 40 years after the initial discovery of EBV, an exact mechanism, explaining the EBV persistence and lymphomagenesis are only addressed partially, we aimed at dissecting the pathways EBV uses to enter the host and how EBV may serve as an oncogenic factor/cofactor in the development of EBV-associated lymphomas.

To answer these questions, we formulated the following working hypothesis:

1. Memory B cells, the main reservoir of EBV *in vivo* are directly susceptible to EBV infection
2. EBV preferentially infects those memory B cells that originate in the nasopharynx-associated lymphatic tissue, the portal of entry of EBV
3. The Follicular Dendritic cell network of the tonsils facilitates infection of memory B cells by EBV
4. Memory B cells retain their characteristic gene expression profile following EBV growth transformation rendering them a more likely target for EBV primary infection than naïve B cells
5. Immune activation, which is a prerequisite for the generation of EBV-associated lymphomas *in vivo*, influences the balance of EBV latent and lytic infection leading to an increased likelihood of EBV-associated lymphomagenesis
6. Toll-like receptors, which are initiating a MyD88-dependent NF-κB activation are expressed at distinct stages during B cell maturation and are responsible for increased plasma cell immunoglobulin production

5. Results

5.1. Distinct *ex vivo* Susceptibility of B-cell subsets to EBV infection According to Differentiation Status and Tissue origin

Marcus Dorner, Franziska Zucol, Christoph Berger, Rahel Byland, Gregory T. Melroe, Michele Bernasconi, Roberto F. Speck, and David Nadal (2008) J. Virol., 82:4400-12.

Abstract

Epstein-Barr virus (EBV) uses tonsils as the portal of entry to establish persistent infection. EBV is found in various B-cell subsets in tonsils but exclusively in memory B cells in peripheral blood. The *in vitro* susceptibilities of B-cell subsets to EBV infection have been studied solely qualitatively. In this work, we examined quantitatively the *in vitro* susceptibilities of various B-cell subsets from different tissue origins to EBV infection. First, we established a centrifugation-based inoculation protocol (spinoculation) that resulted in a significantly increased proportion of infected cells compared to that obtained by conventional inoculation, enabling a detailed susceptibility analysis. Importantly, B-cell infection occurred via the known EBV receptors and infected cells showed EBV mRNA expression patterns similar to those observed after conventional inoculation, validating our approach. Tonsillar naïve and memory B cells were infected *ex vivo* at similar frequencies. In contrast, memory B cells from blood, which represent B cells from various lymphoid tissues, were infected at lower frequencies than their naïve counterparts. Immunoglobulin A (IgA)-positive or IgG-positive tonsillar memory B cells were significantly more susceptible to EBV infection than IgM-positive counterparts. Memory B cells were transformed with lower efficiency than naïve B cells. This result was paralleled by lower proliferation rates. In summary, these data suggest that EBV exploits the B-cell differentiation status and tissue origin to establish persistent infection.

5.2. Integrin expression determines the susceptibility of memory B cell subpopulations to EBV infection

Marcus Dorner, Franziska Zucol, Walter Bossart, Stephan Haerle, Rahel Byland, Christoph Berger, Roberto F. Speck and David Nadal, *manuscript in preparation*

Abstract

The Epstein-Barr virus uses the tonsils as portal of entry to establish life-long persistence within the memory B cell pool. *In vitro* infection of memory B cells leading to the transformation and subsequent immortalization of the cells was employed to show that the resulting lymphoblastoid cell lines showed antigen-specificity for respiratory antigen. Nevertheless, attempts in selecting for non-respiratory antigen-specificity were so far unsuccessful. In this work, we show that memory B cells that were selected via affinity maturation within the nasopharynx-associated lymphatic tissue are susceptible to EBV infection, whereas those that were selected in other secondary lymphatic organs are not. This preferential infection of NALT memory B cells in turn leads to the exclusive transformation of memory B cells with specificity against respiratory pathogens, thereby explaining, why antigen-specific cell lines reacting to non-respiratory antigen could not be generated in the past. The differential infection susceptibility of NALT and non-NALT memory B cells is mediated by the high expression of $\alpha_5\beta_1$ integrin on memory B cells from the NALT, compared to the $\alpha_5\beta_1^{low}$ memory B cells from other lymphatic tissues. EBV uses $\alpha_5\beta_1$ integrin as an attachment factor to facilitate more efficient entry into memory B cells. Upon binding to the integrins, EBV initiates a cellular signalling cascade via *c*-Src and PI3 kinases to activate focal adhesion kinase ultimately leading to enhanced actin filament depolymerization. This in turn enables a more efficient EBV entry process compared to $\alpha_5\beta_1^{low}$ memory B cells.

5.3. Epstein-Barr Virus Triggers Integrin and Focal Adhesion Kinase-Mediated Signalling During Binding to and Infection of Epithelial Cells

Rahel Byland, Marcus Dorner, Michele Bernasconi, David Nadal and Roberto F. Speck, *manuscript in preparation*

Abstract

The γ-herpesvirus Epstein-Barr (EBV) preferentially infects human B and epithelial cells and is associated with a pathogenic role in neoplasia of both types. While EBV infection of B cells is well-characterized, mechanisms of entry into epithelial cells are poorly understood. Binding of the EBV glycoprotein BMRF-2 to integrins of the β1 family plays a crucial role in the epithelial cell infection process. Nevertheless, the mechanisms by which integrins facilitate EBV entry are not defined. We investigated the events triggered by EBV binding to epithelial cells in a tissue culture system using the model cell line AGS. We demonstrate that binding of EBV to integrin and subsequent intracellular signalling are crucial for augmenting EBV infection and that EBV triggers signalling events leading to activation of focal adhesion kinase (FAK). FAK and its downstream effectors PI3K and c-Src were essential for enhancing EBV infection as well as for EBV-mediated changes in the cytoskeleton. EBV binding induced activation of the regulatory small GTPase RhoA leading to a disassembly of cortical actin and the formation of actin stress fibres; furthermore, EBV caused a reorganization and stabilization of microtubuli. These data suggest pathways that may facilitate EBV entry into epithelial cells and further define the EBV-host interaction. A deeper understanding of such mechanisms is key for understanding EBV biology and notably pathogenesis of EBV-associated diseases.

5.4. Follicular Dendritic Cell-B Cell Clusters facilitate the transfer of Epstein-Barr virus to the memory B cell pool

Marcus Dorner, Marianne Tinguely, Franziska Zucol, Christoph Berger, David Nadal, *manuscript in preparation*

Abstract

The Epstein-Barr virus (EBV) is known to exclusively persist in peripheral blood memory B cells following primary infection. This strict tropism for B cells is only eminent for non-lymphoid systems, whereas in the mucosal-associated lymphoid tissue of the Waldeyer's Ring, EBV is also found in cell of non-B-lineage. The life-cycle of EBV holds that it infects B cells at various differentiation stages, i.e. naïve, germinal center and memory B cells. After recirculation of the EBV-infected memory B cells to the tonsil and consecutive activation by an apt antigen, the terminally differentiated EBV-positive Plasma cell will reactivate EBV and shed newly formed virions. Direct viremia of EBV in contrary is hampered by the fast and efficient recognition of EBV lytic cycle antigens by EBV-specific CD8+ T cells. We now report for the first time, that EBV utilizes the antigen-presenting properties of follicular dendritic cells to transfer to B cells establishing persistence without the necessity of free virus. These findings demonstrate how EBV spreads within the host and reveals an additional reservoir for EBV outside the B cell compartment.

5.5. Naïve and memory B cells show distinct gene expression profiles following EBV transformation

Marcus Dorner, Franziska Zucol, Christoph Berger, Michele Bernasconi, Roberto F. Speck, and David Nadal, *manuscript in preparation*

Abstract

The Epstein-Barr virus is known to infect human naïve and memory B cells *ex vivo*, showing preferential infection of memory B cells originating within the nasopharynx-associated lymphatic tissue, the portal of entry of EBV. Even though many reports investigating *in vivo* and *ex vivo* EBV infections exist, the initial target cell for EBV *in vivo* could not yet be identified. As primary infection of EBV cannot be investigated *in vivo* due to lacking small-animal models and primary infection with EBV in humans is rarely detected due to the long incubation period until the onset of clinical symptoms of Infectious mononucleosis we challenged the question, whether the target B cell subpopulation could be identified by comparison of the gene expression signatures of different B cell subpopulations following *ex vivo* infection with EBV. Indeed, we found striking differences in global gene expression when comparing resting naïve B cells and EBV transformed naïve B cells to their memory counterparts. EBV transformation initiates a pronounced upregulation of cancer-associated genes and downregulates pro-apoptotic regulators. In contrast, this dramatic change of gene expression profile is absent in memory B cells, thereby indicating that EBV could use memory B cells as an initial reservoir, as naïve B cells would more likely lead to tumor formation or cell death due to apoposis.

Experimental layout

Naïve and memory B cells were isolated from tonsils as described previously[77]. Resting naïve and memory B cells were subjected to immediate isolation of mRNA, or were infected with EBV by spinoculation and allowed to transform on an autologous B cell-depleted feeder layer for 4 weeks prior to isolation of mRNA. Amplification of cRNA, labelling and hybridization to Affymetrix U133 Plus 2 microarray slides was performed at the Functional Genomics Center Zurich.

Results

We aimed at dissecting the global gene expression changes induced by EBV growth transformation of isolated naïve and memory B cells. For this reason, we isolated naïve and memory B cells from tonsils and either used them for mRNA extraction directly or 4 weeks after EBV growth transformation. Comparing the differences in global gene expression in response to EBV transformation between naïve and memory B cells, we were able to identify genes regulated exceptionally in naïve or memory B cells. Whereas memory B cells preferentially express genes inhibiting extensive cell cycle progression and B cell activation, naïve B cells upregulate a wide panel of genes involved in proliferation and cell cycle progression (Table 5). Furthermore, naïve B cells express a whole pathway associated with tumor growth, tumor vascularisation and lymphomagenesis upon transformation with EBV (Figure 7), whereas this pathway is nearly completely absent in EBV transformed memory B cells (Figure 8).

Table 5. Genes regulated exclusively in naïve B cells following EBV transformation

Biological function	Gene name	Fold change*
Cell adhesion	DNAM-1 (CD226)	36.92
	IRp60 (CD300a)	24.15
	collagen, type V	20.11
	PERP	10.85
	tenascin C	9.58
	β-Parvin	7.65
	discoidin	7.19
	integrin β8	6.93
	P-Selectin	4.54
	ECMR3 (CD44)	2.00
Apoptosis	death-associated protein kinase 1	24.44
	egl nine homolog 3 (C. elegans)	17.10
	homeodomain interacting protein kinase 2	16.70
	lectin, galactoside-binding, (galectin 1)	10.85
	PERP, TP53 apoptosis effector	10.85
	Interleukin-1β	7.27
	tumor necrosis factor receptor 21	2.13
Cell cycle / Proliferation	vascular endothelial growth factor C	17.22
	cyclin D2	14.74
	myosin VI	14.08
	tumor necrosis factor receptor 11a	12.11
	par-6 partitioning defective 6 homolog γ	9.37
	hepatoma-derived growth factor	7.27
	septin 8	5.54
	SLAMF1	4.43
	cyclin-dependent kinase 6	2.52
DNA repair	polymerase (DNA directed), eta	-2.00
	Splicing factor proline/glutamine-rich	-2.52
	chromosome 2 open reading frame 13	-2.52
	protein phosphatase 1, subunit 9B	-4.00
	8-oxoguanine DNA glycosylase	-5.20
B cell differentiation	Fc receptor-like A	-3.67
	B-cell scaffold protein with ankyrin repeats 1	-12.70
	B-cell CLL/lymphoma 6	-76.25

* unpaired t-test; uneven variencies; p<0.01

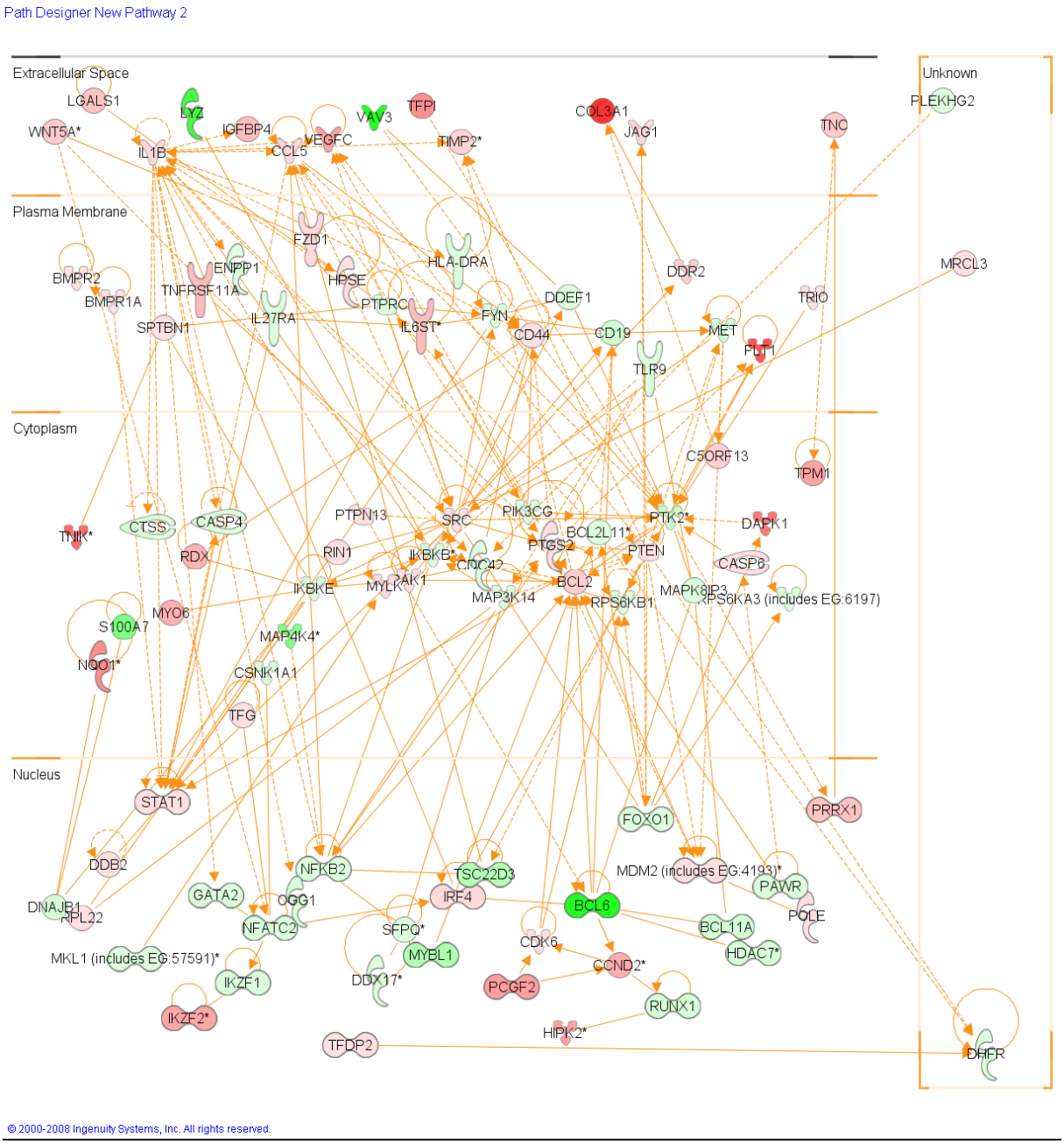

Figure 7. Regulation of Cancer-associated genes in naïve B cells following EBV transformation. Downregulated genes are depicted in green, upregulated genes in red. Analysis was done using Genespring 9.0 and Ingenuity Pathway Analysis. Statistics were performed using nonparametric t-tests assuming uneven variencies and a cutoff of $p<0.01$.

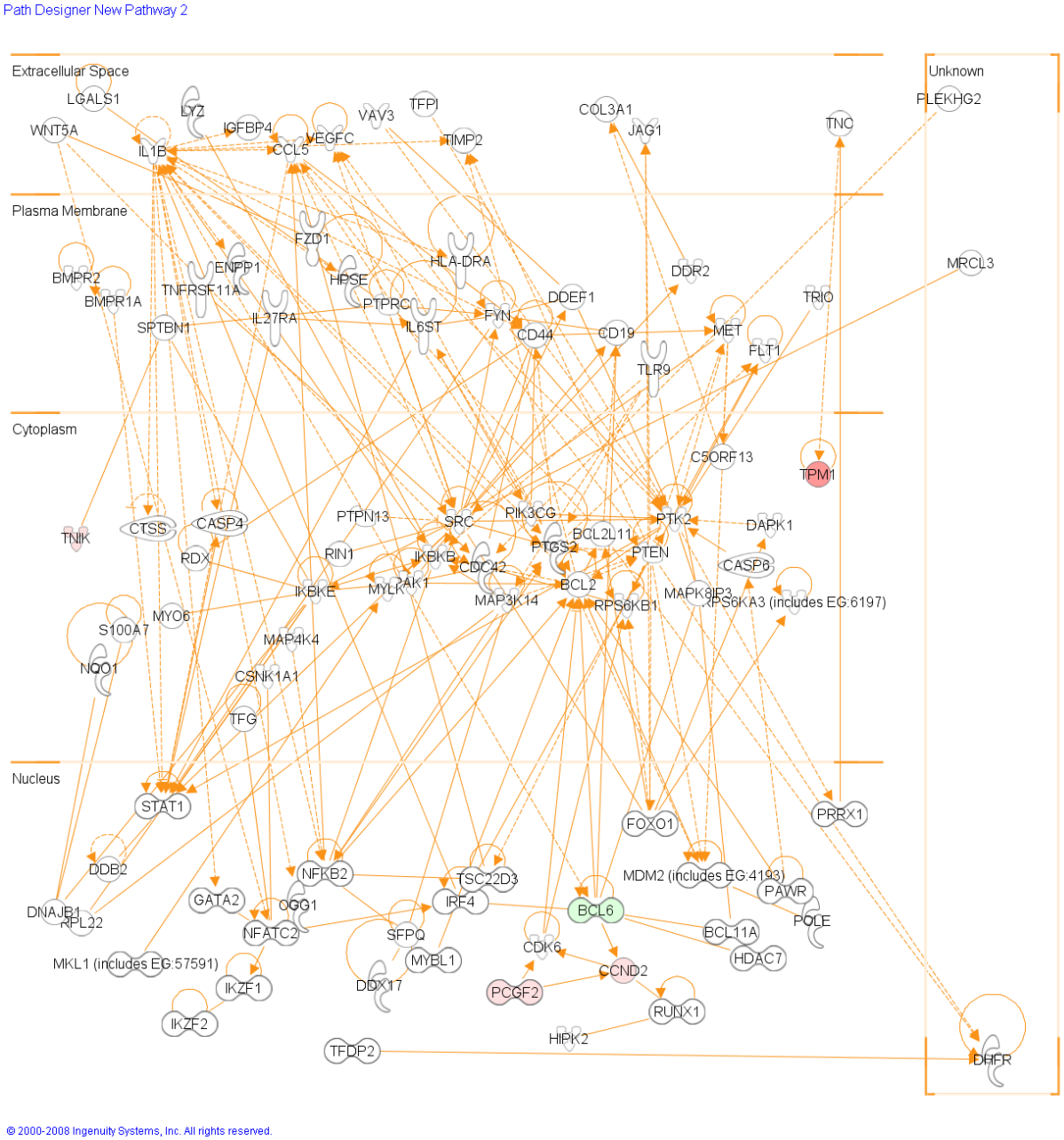

Figure 8. Regulation of Cancer-associated genes in memory B cells following EBV transformation

5.6. Immune activation suppresses initiation of lytic Epstein-Barr virus infection

Kristin Ladell*, Marcus Dorner*, Ludwig Zauner, Christoph Berger, Franziska Zucol, Michele Bernasconi, Felix K. Niggli, Roberto F. Speck, David Nadal (2007) Cell. Microbiol. 9:2055–2069.

* authors contributed equally

Abstract

Primary infection with Epstein-Barr virus (EBV) is asymptomatic in children with immature immune systems but may manifest as infectious mononucleosis, a vigorous immune activation, in adolescents or adults with mature immune systems. Infectious mononucleosis and chronic immune activation are linked to increased risk for EBV-associated lymphoma. Here we show that EBV initiates progressive lytic infection by expression of *BZLF-1* and the late lytic genes *gp85* and *gp350/220* in cord blood mononuclear cells (CBMC) but not in peripheral blood mononuclear cells (PBMC) from EBV-naive adults after EBV infection *ex vivo*. Lower levels of proinflammatory cytokines in CBMC, used to model a state of minimal immune activation and immature immunity, than in PBMC were associated with lytic EBV infection. Triggering the innate immunity specifically via Toll-like receptor-9 of B cells substantially suppressed *BZLF-1* mRNA expression in acute EBV infection *ex vivo* and in anti-IgG-stimulated chronically latently EBV-infected Akata Burkitt lymphoma cells. This was mediated in part by IL-12 and IFN-γ. These results identify immune activation as critical factor for the suppression of initiation of lytic EBV infection. We hypothesize that immune activation contributes to EBV-associated lymphomagenesis by suppressing lytic EBV and in turn promotes latent EBV with transformation potential.

Author contributions: MD performed the experiments described in Figures 1, 2, 3, 6 D-F and 7 and designed the experiments outlined in Figure 6D-F and 7.

5.7. The Toll-like receptor (TLR)10 mRNA expression pattern at distinct B-cell developmental stages is different from that of TLR1-TLR9

*Simone Brandt,[1] *Marcus Dorner,[1] Marianne Tinguely,[3] Franziska Zucol,[1] Jean-Pierre Bourquin,[2] Ludwig Zauner,[1] Christoph Berger,[1] Michele Bernasconi,[1] Roberto F. Speck,[4] and David Nadal[1] (submitted)

* authors contributed equally

Abstact

Toll-like receptors (TLRs) are key receptors of the innate immune response and show cell subset specific expression. We hypothesized that expression and function of TLRs are tailored to distinct stages of B-cell development. We investigated the mRNA expression of TLR genes in B-cells at distinct developmental stages. Hematopoietic stem cells (HSC) and plasma cells were unique by their unrestricted expression of TLR1-TLR9, but absence of TLR10. Triggering plasma cells with TLR ligands augmented immunoglobulin production. By contrast, naïve and memory B-cells lacked TLR3, TLR4 and TLR 8 but expressed all other TLRs. Likewise the expression pattern observed in malignant cells was largely retained compared to their non-transformed developmental stage counterpart. Given the property of TLR1-TLR9 to augment immunoglobulin production upon pathogen recognition, the presence of TLR10 on B-cells rather than plasma cells and HSC and the lack of a TLR10 agonist may point to a unique function of TLR10 including homing.

Author contributions: MD performed the experiments described in Figures 1, 3 and 4 and designed the experiments.

6. Discussion and Outlook

As we could show, memory B cells originating from the lymphatic tissue of the Waldeyer's ring are directly susceptible to EBV infection and contribute to the rapid establishment of persistence after primary infection.

The direct infection of antigen-selected memory B cells holds the advantage, that these cells are readily stimulated via cognate antigen. Previous models based on the initial infection of naïve or germinal center B cells with EBV inevitably lead to the generation of memory B cells that do not recognize apt antigen. Therefore, these EBV-infected "phenotypic" memory B cells fail to undergo terminal differentiation to plasma cells which in turn leads to the inability of EBV to reactivate lytic replication. Another advantage of the model of a direct infection of memory B cells with EBV is that, upon antigenic stimulation, one part of the memory B cells will undergo terminal differentiation whereas the other part will start rapid proliferation ensuring further persistence of EBV within the pool of long-lived memory B cells.

Furthermore, we could show that only those memory B cells that have been generated by respiratory antigen within the Waldeyer's ring tissue are susceptible to EBV infection and transformation. As the tonsils are the portal of entry of EBV, the virus may have adapted to infecting specifically those memory B cells that are specific for respiratory antigen or pathogens. We could also identify the cellular factor restricting the infection susceptibility of non-NALT memory B cells to be β_1 integrin. This has already been shown in the past to play a role in the infection of epithelial cells by EBV. Furthermore, integrins are known as homing receptors specifically guiding the cells to distinct lymphatic tissues. Thereby, EBV uses only those memory B cells that will home back to the tonsils in response to a respiratory infection as reactivation of EBV within the tonsil will facilitate easy spread of EBV to new hosts.

As we could show a direct contribution of memory B cells from the tonsil to EBV persistence, we challenged the question, how infectious EB virions might be delivered to them within the tonsil. Since lytic replication of EBV in plasma cells would elicit a fast immune response mainly by CD4 and CD8 T cells, we investigated the role of possible bystander cells in the infection of B cells with EBV. To evade this, EBV makes use of the Follicular Dendritic cell network present within the GC. EBV is able to bind to FDC which then in turn transfer the virus preferentially to memory B cells homing back to the GC for antigenic reselection.

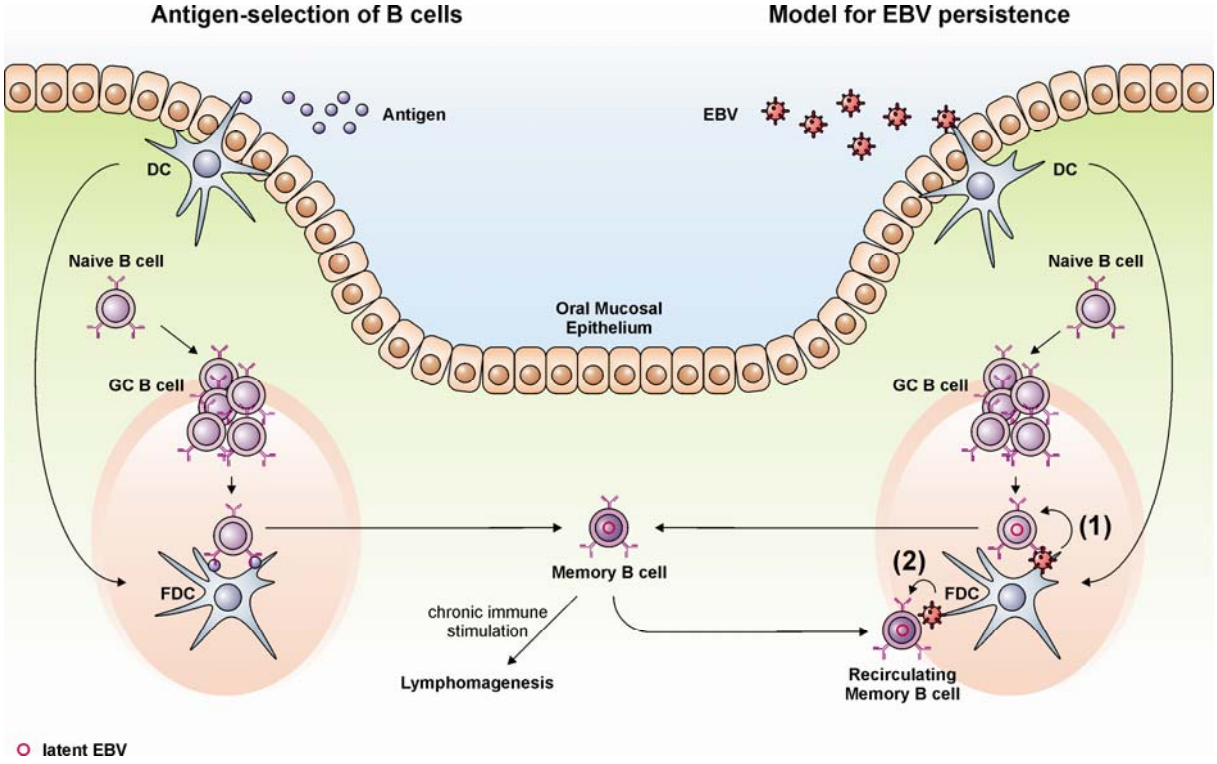

Figure 9. Proposed model for the establishment of EBV persistence and the origin of EBV-associated lymphoma. EBV exploits the normal B cell differentiation pathway by taking the role of foreign antigen. After binding to intraepithelial dendritic cells within the tonsil[121,122], the DC home to the secondary lymphoid follicle. Here, two possibilities exist finally leading to EBV infection of B cells. (1) In the course of positive selection within the GC, EBV may be transferred from the DC to a naïve or GC B cell or (2) EBV persists on the DC and is then transferred to a memory B cell that is homing back to the GC for antigen-reselection. Both models explain why continuous stimulation of EBV-specific CTL does not occur within the tonsil as they do not have the necessity for free viral particles.

This finding opens a new field for targeted drug treatment of IM, as influencing a potential reservoir for EBV by blocking attachment of EBV to FDC might limit fast expansion of EBV-infected B cells, thereby limiting the immune hyperactivation observed during IM.

Additionally, we challenged the question which B cell subpopulation is the initial target for EBV by comparing the genome-wide gene expression profiles of naïve and memory B cells following EBV transformation and could identify a panel of cancer-associated and apoptosis-related genes that are upregulated exclusively in naïve B cells. In contrast, memory B cells downregulate genes that are associated with extensive proliferation and further differentiation. These results allow the speculation, whether naïve B cells are playing a role in the establishment of EBV persistence *in vivo* at all or whether EBV-infected naïve B cells undergo immediate apoptosis to prevent oncogenic transformation.

Dissection of the possible pathways by which EBV establishes persistence within the host enabled us to move on to a closer examination of how EBV could contribute to its associated malignancies, especially those that are also associated with increased immune activation.

As EBV-associated Burkitt's lymphoma is closely associated with endemic malaria, which leads to a chronic stimulation of the immune system, we tested, whether the initial immune activation status has an impact on EBV persistence.

We were able to demonstrate that a higher immune activation status, mainly due to elevated levels of interleukin-12 and interferon γ leads to an inhibition of EBV lytic replication. Furthermore, stimulation of TLR9, which is also constitutively triggered during chronic malaria, resulted in an IL-12/IFN-γ-dependent inhibition of EBV replication. This clearly demonstrates that the innate immune system which senses a wide array of different pathogens directly influences the life-cycle of EBV resulting in a more restricted latency of EBV-infected B cells. A Latent infection of EBV-infected B cells in turn is mandatory for the development of EBV-associated malignancies such as Burkitt's lymphoma.

Thus, our findings regarding the differential susceptibility of B cell subpopulations to EBV infection and the impact of immune activation of EBV life-cycle serve to a better understanding of EBV-associated lymphomagenesis.

The next milestones that need to be further determined are on the one hand, whether the direct infection of antigen-specific memory B cells by EBV by the highly efficient spinoculation protocol holds the key to the development of a patient- and pathogen-specific antibody therapy, as EBV-transformed memory B cells could be generated, secreting antibodies with specificity for the whole pool of antigens the host encountered so far.

The second important step would include validation of $\alpha_5\beta_1$ integrin or the so far unidentified attachment factor of EBV to FDC as a drugable target for vaccination or even treatment against EBV persistence. Blocking the entry of EBV either into the whole B cell pool or specifically into memory B cells holds the potential to prevent the establishment of EBV persistence within the memory B cell pool even after seroconversion.

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8. Manuscripts

1. Marcus Dorner, Franziska Zucol, Christoph Berger, Rahel Byland, Gregory T. Melroe, Michele Bernasconi, Roberto F. Speck, and David Nadal (2008) "Distinct ex vivo Susceptibility of B-cell subsets to EBV infection According to Differentiation Status and Tissue origin". *J. Virol.*, 82:4400-12.
2. Marcus Dorner, Franziska Zucol, Walter Bossart, Stephan Haerle, Rahel Byland, Christoph Berger, Roberto F. Speck and David Nadal "Integrin expression determines the susceptibility of memory B cell subpopulations to EBV infection", *manuscript in preparation.*
3. Rahel Byland, Marcus Dorner, Michele Bernasconi, David Nadal and Roberto F. Speck, "Epstein-Barr Virus Triggers Integrin and Focal Adhesion Kinase-Mediated Signalling During Binding to and Infection of Epithelial Cells", *manuscript in preparation.*
4. Kristin Ladell[*], Marcus Dorner[*], Ludwig Zauner, Christoph Berger, Franziska Zucol, Michele Bernasconi, Felix K. Niggli, Roberto F. Speck, David Nadal (2007) "Immune activation suppresses initiation of lytic Epstein-Barr virus infection". *Cell. Microbiol.* 9:2055–2069.
5. Simone Brandt[*], Marcus Dorner[*], Marianne Tinguely, Franziska Zucol, Jean-Pierre Bourquin, Ludwig Zauner, Christoph Berger, Michele Bernasconi, Roberto F. Speck, and David Nadal. "The Toll-like receptor (TLR)10 mRNA expression pattern at distinct B-cell developmental stages is different from that of TLR1-TLR9" (submitted). *submitted*.

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Distinct Ex Vivo Susceptibility of B-Cell Subsets to Epstein-Barr Virus Infection According to Differentiation Status and Tissue Origin▽

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Epstein-Barr virus (EBV) uses tonsils as the portal of entry to establish persistent infection. EBV is found in various B-cell subsets in tonsils but exclusively in memory B cells in peripheral blood. The in vitro susceptibilities of B-cell subsets to EBV infection have been studied solely qualitatively. In this work, we examined quantitatively the in vitro susceptibilities of various B-cell subsets from different tissue origins to EBV infection. First, we established a centrifugation-based inoculation protocol (spinoculation) that resulted in a significantly increased proportion of infected cells compared to that obtained by conventional inoculation, enabling a detailed susceptibility analysis. Importantly, B-cell infection occurred via the known EBV receptors and infected cells showed EBV mRNA expression patterns similar to those observed after conventional inoculation, validating our approach. Tonsillar naïve and memory B cells were infected ex vivo at similar frequencies. In contrast, memory B cells from blood, which represent B cells from various lymphoid tissues, were infected at lower frequencies than their naïve counterparts. Immunoglobulin A (IgA)-positive or IgG-positive tonsillar memory B cells were significantly more susceptible to EBV infection than IgM-positive counterparts. Memory B cells were transformed with lower efficiency than naïve B cells. This result was paralleled by lower proliferation rates. In summary, these data suggest that EBV exploits the B-cell differentiation status and tissue origin to establish persistent infection.

Epstein-Barr virus (EBV) infects over 90% of the world's population and establishes life-long persistence in the memory B cells of the infected host (32). In vitro, EBV can transform and immortalize B cells to generate lymphoblastoid cell lines (LCLs) (19). That characteristic points at the potential neoplastic role of EBV in several tumors of B-cell origin in humans, including Burkitt's lymphoma, Hodgkin's disease, and posttransplant lymphoproliferative disease (8, 21).

The mucosal lymphoid tissues of the Waldeyer's ring, including tonsils, act as the original site of EBV infection and the reservoir for the virus where B-cell infection initiates during infectious mononucleosis (2–4, 24, 28). EBV is detected in both naïve and memory B cells when infected tonsillar tissue is examined (3). By contrast, EBV is found exclusively in memory B cells when peripheral blood is examined (2, 18). Notably, EBV-infected naïve B cells isolated from tonsils express a lymphoblastoid phenotype corresponding to latent EBV genes, whereas EBV-infected memory B cells from tonsils express a more restricted pattern (3). In contrast, the EBV-infected memory B cells isolated from the peripheral blood display a very restricted pattern of EBV latent gene expression, in which no latent EBV genes, with the possible exception of *LMP2*, are expressed (3). Thus, B cells from distinct compartments show different EBV infection and gene expression patterns. Notably, the cited data from in vivo studies are purely descriptive and no data on the relative EBV susceptibilities of naïve and memory B cells from different compartments are yet available. In particular, we do not know to what extent the pool of EBV-infected memory B cells in the tonsils is derived from naïve EBV-infected B cells that have gone through a germinal-center reaction or how the pools of infected cells are influenced by distinct proliferation rates of naïve and memory B cells following EBV infection. Also, given the different EBV gene expression patterns in naïve and memory tonsillar B cells, EBV-infected cells from different subsets may be subjected to differential recognition by EBV-specific immune responses, which in turn may impact the survival and thus the frequencies of these cells. Information on the relative susceptibilities of the various B cells and the subsequent phenotypic changes would be highly desired for a detailed understanding of EBV pathogenesis.

There are only limited detailed quantitative data available about the susceptibilities to EBV infection of distinct B-cell subsets. It appears that EBV infects various subsets of B cells from tonsils, adenoids, or peripheral blood in vitro (3, 6, 11, 15). In very early studies, the general susceptibility of B cells to EBV infection in vitro was demonstrated by assessing transformation rates of nonseparated (6) or isolated (15) B cells. Transformation, however, provides only indirect information about EBV infectivity and does not take into account the proliferation and apoptosis rates of EBV-infected B-cell subsets. More recent studies isolated tonsillar or adenoidal B cells on the basis of CD10, CD77, and/or surface immunoglobulin isotype expression (3, 11). Nowadays, memory B cells can be more accurately identified thanks to the specific memory B-cell

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marker CD27 (20, 41). Most importantly, published work on the in vitro infection of B-cell subsets has addressed the issue of susceptibility to EBV infection only qualitatively, and the efficiencies of infection of B-cell subsets from distinct tissues have not been studied in detail. However, quantitative differences in susceptibilities of B cells to EBV infection may depend on the tissue origin, and the elucidation of these differences will give further insight into EBV pathogenesis.

The aim of this work was to assess the efficiencies of EBV infection of distinct B-cell subsets from different lymphoid compartments and to compare the transformation behaviors of B-cell subsets infected ex vivo with EBV. To enable these investigations, we established a centrifugation-based inoculation protocol, so-called spinoculation, using a recombinant EBV encoding enhanced green fluorescent protein (EGFP) (39, 40). This procedure resulted in significantly higher proportions of cells infected ex vivo than conventional inoculation and enabled us to identify EBV-infected B-cell subsets as early as 24 h after infection and to characterize EBV-induced proliferation of naïve and memory B cells. Since EBV-specific immune responses may impact the cellular infection and transformation processes, we used primary cells exclusively from EBV-naïve individuals.

MATERIALS AND METHODS

Cell culture. The EBV-infected marmoset cell lines B95.8 (27) and B95.8EBfaV-GFP (39) were maintained in RPMI 1640 medium (Gibco, Basel, Switzerland) with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin, referred to hereafter as complete medium. Primary cells were cultivated in complete medium supplemented with 1 μM sodium pyruvate and 1× nonessential amino acids (Gibco).

Isolation of mononuclear cells. Primary human mononuclear cells were isolated from palatine tonsils obtained from EBV-seronegative patients undergoing routine tonsillectomy or from blood samples taken from healthy EBV-seronegative adult donors. Tonsillar mononuclear cells (TMC) were prepared as described previously (14). Briefly, tonsils were disintegrated with a scalpel in complete medium and passed through a 70-μm-pore-size cell strainer (Falcon, Wohlen, Switzerland). For the isolation of B-cell subsets, TMC were further purified by density gradient centrifugation with Ficoll-Hypaque (GE Healthcare, Stockholm, Sweden). Peripheral blood mononuclear cells (PBMC) were purified by Ficoll-Hypaque density gradient centrifugation according to the instructions of the Ficoll-Hypaque manufacturer. The viability of primary cells, as determined by trypan blue exclusion, was >99% in all preparations. The EBV serostatuses of the mononuclear cell donors were determined by using the Immunodot Mono G and Mono M kit according to the instructions of the manufacturer (Ruwag Diagnostics, Bettlach, Switzerland). Informed consent was obtained from subjects or parents before the study. The institutional ethics committee approved the collection and use of clinical material.

Preparation of virus stock. B95.8 and B95.8EBfaV-GFP cells were seeded at a density of 10^6 cells/ml and were stimulated to release virus by being cultured for 4 days in complete medium containing 50 ng of 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Sigma-Aldrich, Buchs, Switzerland)/ml. Cell suspensions were centrifuged at 400 × *g* for 5 min. Supernatant was passed through a 0.45-μm-pore-size cellulose acetate filter (Millipore, Zug, Switzerland) and stored at −80°C.

Infection of primary cells. EBV inoculation was done as described earlier (23). Briefly, 1 ml of supernatant from EBV-producing cell lines was added to 2×10^5 cells in 1 ml of complete medium for 3 h at 37°C. For so-called conventional inoculation, supernatants from TPA-induced EBV-producing cells lines were used. Cell suspensions were centrifuged at 300 × *g* for 5 min, and cells were resuspended in fresh complete medium.

For spinoculation, EBV-containing supernatants were concentrated using a Vivaspin 20 concentrator with polyethersulfone and a molecular weight cutoff of 1,000,000 according to the instructions of the manufacturer (Sartorius-Stedim, Dietikon, Switzerland). Primary cells were centrifuged at 300 × *g* for 5 min, and the medium was completely replaced by the concentrated virus-containing supernatants to a final concentration of 10^6 cells/ml. The cell suspensions were then centrifuged for 1 h at 800 × *g* at 24°C. After spinoculation, cells were washed in phosphate-buffered saline and resuspended in fresh complete medium.

Flow cytometry. Flow cytometry was carried out on a Cytomics FC500 instrument (Beckman Coulter, Nyon, Switzerland) with FlowJo software, used in accordance with the instructions of the manufacturer (Treestar, Ashland, OR).

Antibodies. Fluorochrome-conjugated monoclonal antibodies directed against human CD19, CD21, CD27, HLA-DR, HLA-DP, HLA-DQ, immunoglobulin D (IgD), IgM, IgG, and IgA were purchased from BD Pharmingen (a division of BD Biosciences, Basel, Switzerland). Unconjugated and fluorescein isothiocyanate (FITC)-conjugated antibodies directed against EBV gp350/220 were purchased from Chemicon. Anti-EBV gp42 was a generous gift from L. Hutt-Fletcher (Shreveport, LA). Rabbit antibodies against poly(ADP-ribose) polymerase (PARP) and actin were from Cell Signaling Technology (Danvers, MA).

Inhibition of EBV infection. Concentrated EBV-containing supernatants were treated with either anti-gp350/220, anti-gp42, or a combination of the two antibodies at dilutions of 1:10, 1:100, and 1:1,000 at 4°C for 1 h. TMC were infected with the concentrated supernatants by spinoculation at 4°C to prevent virus entry and stained with FITC-anti-gp350/220, and the amount of bound virus was determined by flow cytometry. TMC were also analyzed for the expression of EGFP 24 h after inoculation to evaluate the number of EBV-infected cells. Furthermore, TMC were pretreated with anti-CD21 and anti-HLA-DR, anti-HLA-DP, and anti-HLA-DQ at dilutions of 1:10, 1:100, and 1:1,000 for 1 h at 4°C before inoculation with EBV by spinoculation. TMC were analyzed for EBV infection 24 h after spinoculation by flow cytometric quantification of EGFP expression.

Isolation of B-cell subpopulations. B cells were isolated using the B-cell isolation kit II according to the instructions of the manufacturer (Miltenyi Biotech, Bergisch Gladbach, Germany). Further separation into naïve and memory B cells was performed using the naïve-B-cell isolation kit (Miltenyi Biotech) or CD27 microbeads (Miltenyi). IgM-positive B cells were isolated by treating B cells with phycoerythrin-conjugated anti-human IgM and separating the cells with the PE multisort kit (Miltenyi). To obtain IgM-positive memory B cells, cells were further subjected to CD27 microbead isolation. The purity of isolated B-cell subsets was determined by flow cytometry using antibodies to human CD19, CD27, IgD, IgM, IgG, and IgA for discrimination between naïve and memory B cells. Purified cell populations used for experiments were always >95% pure.

RNA extraction and quantitative real-time PCR. The preparation of total RNA was performed using an RNeasy mini kit according to the instructions of the manufacturer (Qiagen, Hombrechtikon, Switzerland). After DNase I digestion (Ambion, Rotkreuz, Switzerland), RNA was reverse transcribed using an Omniscript reverse transcription-PCR kit (Qiagen). Quantitative PCR for EBV gene mRNA was performed using specific primers and probes for *EBNA2*, *EBNA1*, *EBNA3A*, *EBNA3C*, *LMP1*, *LMP2*, or *BZLF1* as described previously (5, 23, 34). All reactions were performed on a real-time PCR machine (ABI Prism 7700; Applied Biosystems) with TaqMan mastermix (Eurogentec, Seraing, Belgium) by using *HMBS* as a housekeeping gene. Cycling conditions were as follows: a 10-min denaturation step at 95°C was followed by 40 cycles of denaturation for 15 s at 95°C and annealing and synthesis for 1 min at 60°C.

Transformation assay. Transformation efficiency was assessed by a modification of the classical protocols (35). Briefly, either unseparated TMC or isolated naïve and memory B cells were infected with B95.8 EBV by either conventional inoculation or spinoculation. The wild-type B95.8 EBV was used to exclude potential interference of the recombinant EBV with the transformation process. To provide similar culture conditions for purified B cells and mononuclear cells, we added B-cell-depleted TMC as a feeder layer for ex vivo EBV-infected naïve and memory B cells immediately after spinoculation (29). One half of the medium was changed every 3 days for 4 weeks in all wells initially used.

Distributions of naïve and memory B cells were evaluated by flow cytometry 1, 2, 3, and 4 weeks after infection. After 4 weeks, wells still showing proliferation were suggested to contain transformed B cells (35). At that time, the proportion of B cells in all measured samples was above 98%. The transformation efficiency was calculated by counting the wells that contained actively proliferating B cells 4 weeks following infection.

Western blotting. For Western blotting, whole-cell extracts were prepared from 10^6 cells with RIPA buffer (50 mM Tris-Cl, pH 6.8, 100 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate) supplemented with complete mini protease inhibitor cocktail (Roche Applied Sciences, Rotkreuz, Switzerland) and 1 mM sodium orthovanadate (Sigma-Aldrich) for 20 min on ice. For the detection of primary antibodies, we used horseradish peroxidase-labeled goat anti-rabbit or anti-mouse antibodies from Pierce Biotechnology (Rockford, IL). Signal detection was performed by using the chemiluminescence substrate from Pierce and scanning directly with the ChemiGenius imaging system (SynGene,

Cambridge, United Kingdom), and signals were quantified with the GeneTools 3.1 image analysis software (SynGene).

ATP uptake assay. For ATP uptake, naïve and memory B cells were seeded at a density of 0.8×10^6 cells per ml in complete medium and were allowed to proliferate for 24 h. ATP uptake was measured using the ViaLight bioassay kit according to the instructions of the manufacturer (Lonzabiosciences, Basel, Switzerland). As a reference, a standard curve was used to determine the absolute cell numbers.

Statistical analyses. All experiments were carried out at least three times, and representative results from the experiments are presented. Statistical analyses of significance (P values) were based on a two-tailed paired t test. P values of <0.05 were regarded as statistically significant.

RESULTS

Spinoculation with concentrated virus substantially increases the proportion of primary B cells infected by EBV ex vivo. We first established an infection protocol that would provide a sufficient number of infected cells for analysis. Standard ex vivo inoculation protocols for primary human mononuclear cells yield proportions of EBV-infected cells of up to 2% of total B cells (40). We thus adapted a protocol originally designed to infect T cells with human immunodeficiency virus type 1 that is based on low-speed centrifugation of cells in human immunodeficiency virus type 1-containing supernatants (spinoculation) (31). The low-speed centrifugation is thought to facilitate virus binding to the cells, in contrast to pelleting of the virus (31). To rapidly identify EBV-infected cells, we used EBfaV-GFP, a recombinant EBV carrying a gene for EGFP that replaces the *LMP2* gene (39). The cytomegalovirus immediate-early promoter is active in naïve and memory B cells (22), drives the constitutive expression of EGFP (40), and thus allows the identification of all EBV-infected B cells as early as 24 h postinfection (40).

Following ex vivo inoculation of TMC with supernatants from the EBV-producer cell line EBfaV-GFP, <1% of B cells were infected at 48 h (Fig. 1A). Conventional inoculation using supernatants from TPA-induced EBfaV-GFP infected up to around 2% of B cells, even if the supernatants were concentrated 50-fold. Spinoculation with supernatants from non-TPA-induced EBfaV-GFP infected around 6% of B cells when the supernatants were concentrated 50-fold. The fraction of infected TMC B cells could be further increased to 50% by adding concentrated EBV-containing supernatants from TPA-induced EBfaV-GFP (Fig. 1A to E). Similarly, ex vivo inoculation of PBMC by spinoculation with TPA-induced and concentrated EBV led to 25-fold-higher infection frequencies than inoculation with nonconcentrated EBV by conventional protocols (data not shown). Thus, spinoculation markedly increased the fraction of infected B cells compared to that obtained by conventional inoculation when either TPA-induced or 50-fold-concentrated EBV was used.

Overall, the percentages of EBV-positive cells increased from <1 to >30% of TMC and from 1 to 5% of PBMC. As expected, B cells constituted a vast majority of the infected cells (Fig. 1E).

EBV binding and the infection of B cells after spinoculation depend on viral glycoproteins and their specific receptors. The attachment of EBV to B cells is mediated by the direct interaction of EBV gp350/220 with cellular CD21, initiating receptor-mediated endocytosis. After binding to CD21, EBV gp42 can interact with host HLA class II, leading to a conformational change in the viral glycoproteins and triggering fusion with the host cell membrane (16, 38). To exclude nonspecific EBV uptake during spinoculation (42, 43), we treated the EBV-containing supernatants with antibodies against EBV gp350/220, gp42, or both. In addition, we treated the target B cells with antibodies against CD21, HLA class II, or both before spinoculation (Fig. 1F and G). Cells were kept at 4°C during spinoculation to inhibit EBV entry (30).

To quantify the efficiency of EBV binding to cells, we stained cell-associated EBV with FITC-conjugated anti-EBV gp350/220 antibodies and examined the cells by flow cytometry (Fig. 1F). Blocking EBV glycoprotein gp350/220 reduced the mean efficiency of binding of spinoculated EBV to B cells to 65% in a dose-dependent manner, as also observed earlier (26). Blocking the fusion-triggering EBV gp42 also resulted in a loss of binding efficiency to 50% of that in controls, which may be due to sterical hindrance or the loss of the gp350/220-CD21 interaction with the use of blocking antibodies to gp42. Simultaneous blocking of gp350/220 and gp42 reduced the mean binding efficiency of EBV to 22% of that in untreated samples, showing that both attachment via gp350/220 and the subsequent interaction between gp42 and HLA class II are required during the infection of cells by spinoculation (Fig. 1F). Blocking of the EBV receptor and coreceptor with anti-CD21 or anti-HLA class II antibodies reduced EBV attachment to 10 to 15% of that in controls, and the simultaneous use of both antibodies further reduced the mean level of cells showing cell-associated EBV to <10% (Fig. 1F). The blocking of EBV binding by anti-EBV gp350/220 did not influence the detection of bound EBV by FITC–anti-EBV gp350/220 in comparisons of EBV with free gp350/220 to EBV with blocked gp350/220 (Fig. 1F).

Similarly, blocking either gp350/220 or gp42 or both by monoclonal antibodies dose dependently decreased the mean levels of infected B cells from 40 to 50% to less than 5% (Fig. 1G). Blocking CD21, HLA class II, or both dose dependently reduced the mean levels of infected B cells from 40 to 50% to 10 to 20% (Fig. 1G). These results indicate that spinoculation does not bypass and change the requirements for the normal binding and fusion of EBV with B cells.

Infecting lymphoid cells with EBV by spinoculation results in a type III EBV gene mRNA expression pattern. Normally, following the ex vivo infection of B cells by conventional inoculation, EBV undergoes the so-called type III program of latency gene expression (33). To determine if spinoculation has no aberrant effects on EBV gene expression after infection, we compared the EBV mRNA expression profiles after infection by spinoculation with those after infection by conventional inoculation. Indeed, patterns of mRNA expression of EBV latent genes, including *EBNA1*, *EBNA2*, *LMP1*, and *LMP2*, in TMC and PBMC after conventional inoculation or spinoculation with B95.8 EBfaV-GFP were similar (Fig. 2). Nevertheless, mRNA expression levels after spinoculation were 10- to 50-fold higher than those after conventional inoculation (Fig. 2). This increase is likely explained by the 25- to 50-fold enhancement in the proportion of infected cells following spinoculation compared to that following conventional inoculation (Fig. 1). Thus, EBV mRNA expression patterns were similar irrespective of the inoculation protocol used. This finding sug-

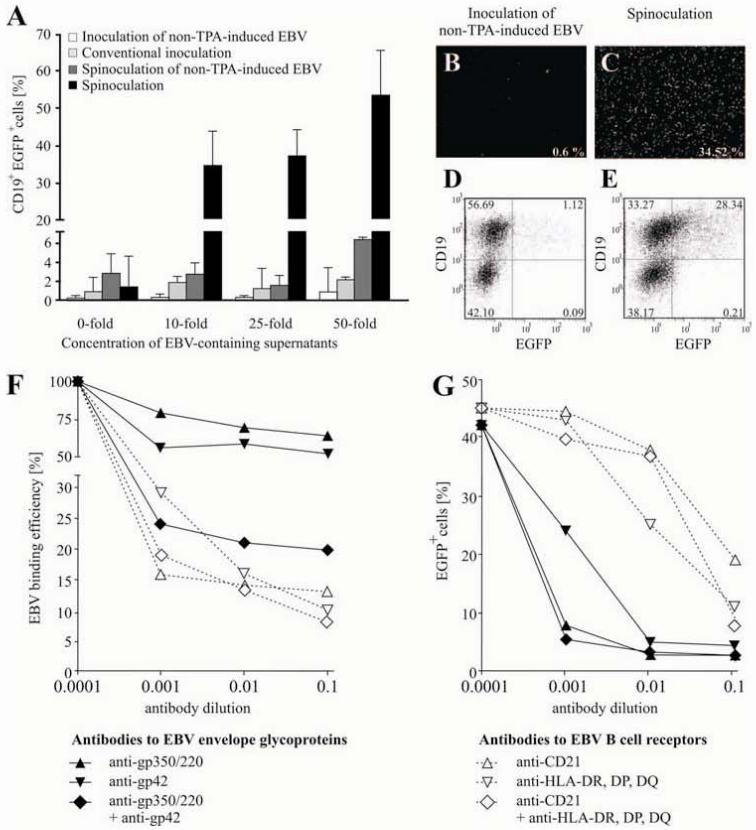

FIG. 1. Spinoculation increases the total numbers of ex vivo EBV-infected B cells without affecting the specificity of infection. (A) Results of inoculation and spinoculation of TMC with different concentrations of TPA-induced or uninduced cultures of the EBV strain B95.8EBfaV-GFP. Cells were counterstained with *R*-phycoerythrin-labeled antibody against the B-cell surface marker CD19 and analyzed by flow cytometry 48 h after inoculation. (B and C) Fluorescence microscopy of TMC 48 h after infection by inoculation with non-TPA-induced supernatants or by spinoculation with 50-fold-concentrated supernatants from TPA-induced cultures of the EBV strain B95.8EBfaV-GFP. The percentages of $EGFP^+$ cells among the total number of cells are presented. (D and E) Flow cytometry quantification of $EGFP^+$ cells 48 h after ex vivo inoculation with non-TPA-induced EBV (D) or spinoculation with 50-fold-concentrated supernatants from TPA-induced cultures of the EBV strain B95.8EBfaV-GFP (E). Cells were counterstained with *R*-phycoerythrin-labeled antibody directed against CD19. Values presented show the mean infection rates of results from four independent experiments. (F) Binding of EBV to B cells. The efficiency of EBV binding to B cells was assessed using 50-fold-concentrated supernatants from TPA-induced B95.8EBfaV-GFP cells. The concentrated supernatants were treated with antibodies against the EBV glycoprotein gp350/220, gp42, or both at serial dilutions or mock treated and used for ex vivo spinoculation of TMC at 4°C. Conversely, TMC were treated with antibodies specifically targeting the two receptors required for EBV entry, CD21 and HLA class II, at serial dilutions (1:10, 1:100, 1:1,000, and 1:10,000) and then subjected to ex vivo spinoculation with 50-fold-concentrated supernatants from TPA-induced B95.8EBfaV-GFP cells. The efficiency of the binding of EBV to B cells was determined by flow cytometry using FITC-labeled anti-EBV gp350/220 immediately after spinoculation. (G) Infection of B cells with EBV. The EBV infection of B cells was quantified by flow cytometry to measure the EGFP fluorescence intensity 24 h after spinoculation. Results from one independent experiment of two are shown.

gested that spinoculation does not elicit an aberrant effect on EBV gene expression.

As in subsequent experiments we planned to use the reporter virus EBfaV-GFP, we wished to determine if it behaved as predicted. Previous studies have reported that the recombinant B95.8EBfaV-GFP infects and transforms B cells as well as the wild-type strain B95.8 (39). With the exception of *LMP2* (which had lower mRNA expression levels after infection with B95.8 EBfaV-GFP), infection with either virus gave the same EBV mRNA expression profile. Although *LMP2* is replaced by *EGFP* in the reporter virus, there is still production of wild-type virus in the producer cells (40), which we believed resulted in the low *LMP2* mRNA expression observed (Fig. 2D).

There is the possibility that the B-cell phenotype has an effect on the EBV mRNA expression pattern, an important feature in the context of the potential transformation ability.

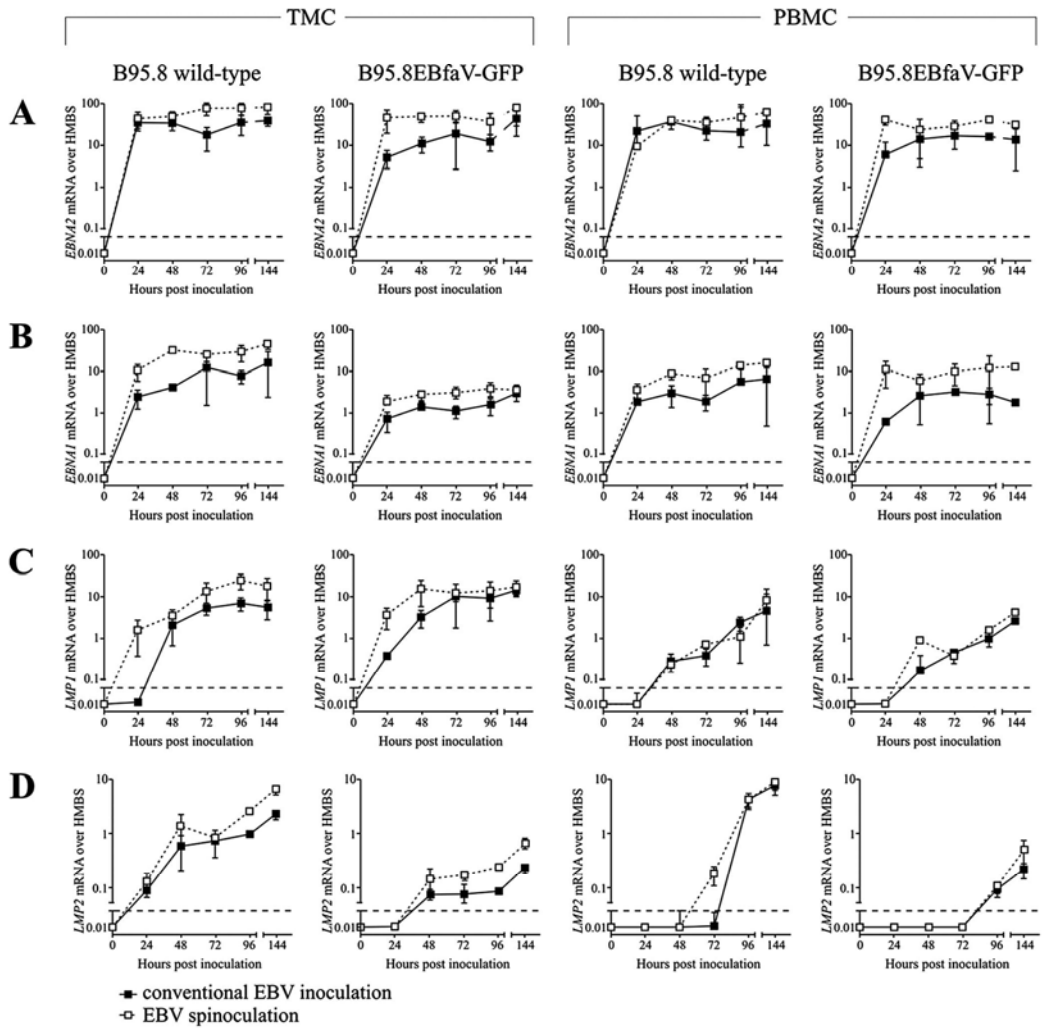

FIG. 2. Spinoculation and conventional inoculation of TMC and PBMC with EBV yielded similar mRNA expression patterns. TMC and PBMC infection by spinoculation results in EBV gene mRNA expression patterns similar to those resulting from infection by conventional inoculation using either the wild-type B95.8 EBV or B95.8EBfaV-GFP. Longitudinal EBV gene mRNA expression was assessed by specific quantitative real-time PCR analysis. The mRNA expression of the genes encoding EBV nuclear antigen, *EBNA2* (A) and *EBNA1* (B), and those encoding the EBV latent membrane proteins, *LMP1* (C) and *LMP2* (D), showed a sequential activation of EBNA2 and EBNA1, followed by LMP1 and LMP2. The increased rate of infection of the cells with EBV after spinoculation was mirrored in the mRNA expression levels of EBV genes. The use of the recombinant B95.8EBfaV-GFP EBV did not influence EBV gene mRNA expression compared to that in wild-type EBV. All EBV gene mRNA expression data were normalized to the mRNA expression of the housekeeping gene for hydroxymethylbilane synthase (*HMBS*). Results shown are from TMC from three donors and are expressed as means ± standard errors of the means (SEM).

We therefore isolated naïve and memory B cells from TMC at 24-h intervals during the first 7 days after spinoculation with EBV and quantified EBV mRNA expression by quantitative real-time PCR. The master regulator of all other EBV latent genes, *EBNA2*, and the latent EBV genes *EBNA1*, *EBNA3A*, *EBNA3C*, *LMP1*, and *LMP2* showed almost identical patterns of expression in naïve and memory B cells (Fig. 3A to F). No expression of the initiator and master regulator gene of lytic infection, *BZLF1*, was detected in either B-cell subset (Fig. 3G). Also, the expression of lytic genes downstream of *BZLF1*

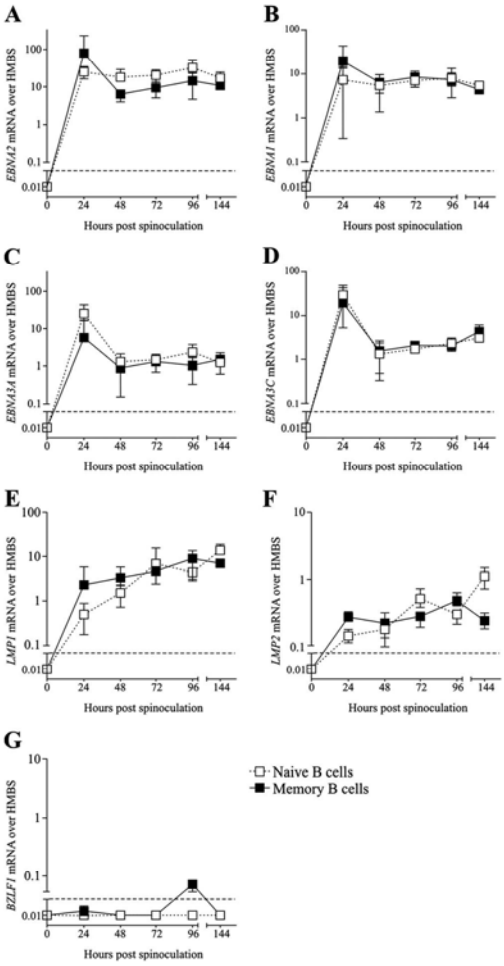

FIG. 3. EBV gene expression patterns are similar in tonsillar memory and naïve B cells after infection. (A to F) Comparison of the longitudinal EBV gene mRNA expression patterns in tonsillar naïve and memory B cells after ex vivo infection by spinoculation. Both memory and naïve B cells expressed the EBV latency genes *EBNA2* (A), *EBNA1* (B), *EBNA3A* (C), *EBNA3C* (D), *LMP1* (E), and *LMP2* (F). (G) Neither B-cell subset initiated EBV lytic infection, as documented by the absence of mRNA expression of the EBV immediate-lytic gene *BZLF1*. After spinoculation of TMC with EBV ex vivo, memory and naïve B cells were isolated with magnetic beads at different times. The purity of the B-cell subsets was >98%. Results shown are from TMC from three donors and are expressed as means ± SEM. HMBS, hydroxymethylbilane synthase mRNA.

was absent in naïve and memory B cells (data not shown). This finding is consistent with those in previously published reports suggesting that EBV manifests primarily as a latent type III infection in TMC after ex vivo infection and verifies that EBV establishes latency in memory B cells in a fashion similar to that in naïve B cells (1). The isolation of naïve B cells and that of memory B cells before EBV infection showed comparable results, thereby excluding possible effects of the cell isolation on the EBV mRNA expression (data not shown).

Memory and naïve B cells from tonsils, but not those from peripheral blood, are equally susceptible to EBV infection. CD21 and HLA-DR are absolutely required for the successful infection of B cells with EBV. Among TMC and PBMC, naïve ($CD19^+$ $CD27^-$) and memory ($CD19^+$ $CD27^+$) B cells showed similar levels of CD21 (Fig. 4A and B) and HLA-DR (Fig. 4C and D) expression (summarized in Fig. 4E and F). Conventional inoculation of TMC (Fig. 4G) or PBMC (Fig. 4H) resulted in very low levels of infected $CD19^+$ B cells (naïve B cells, 0.9% ± 0.1% and 0.5% ± 0.3%, respectively; memory B cells, 0.02% ± 0.01% and 0.01% ± 0.01%, respectively). Spinoculation increased the proportions of infected naïve and memory B cells dramatically compared to those obtained by conventional inoculation. Strikingly, the proportions of infected cells among the B-cell subsets of TMC were rather similar (39.1% ± 2.2% EBV-positive memory B cells versus 49.6% ± 16.7% EBV-positive naïve B cells) (Fig. 4I). However, among PBMC, the proportion of infected naïve B cells (69.2% ± 5.1%) was one-third higher than the proportion of infected memory B cells (25.6% ± 4.8%; $P = 0.016$) (Fig. 4J).

Tonsillar memory B cells are more susceptible to EBV infection after class switching than before. Ehlin-Henriksson et al. reported that tonsillar B-cell subpopulations are equally susceptible to EBV infection in vitro, irrespective of immunoglobulin isotype expression (11). That study did not discriminate between naïve ($CD27^-$) and IgM-expressing memory ($CD27^+$) B cells (44). We therefore characterized in detail the isotype expression in tonsillar memory ($CD27^+$) B cells susceptible to EBV infection. Thus, we isolated memory ($CD27^+$) B cells from TMC and further segregated them into IgM-negative and IgM-positive populations. Forty-eight hours after inoculation with B95.8EBfaV-GFP, we analyzed the infection frequencies among memory B cells expressing IgM, IgA, and IgG (Fig. 5A). We found that IgM-expressing memory B cells showed a significantly lower frequency of EBV infection than memory B cells expressing IgA or IgG, suggesting that class-switched memory B cells are more susceptible to EBV infection than their non-class-switched counterparts. We had to exclude that EBV infection ex vivo alters the relative frequencies of the B-cell subpopulations expressing IgM, IgA, or IgG. To this end, we used isolated tonsillar memory ($CD27^+$) B cells and infected them ex vivo with B95.8EBfaV-GFP. Forty-eight hours after infection, mock-treated and EBV-exposed memory B cells showed similar relative amounts of cells expressing IgM, IgA, or IgG (Fig. 5B).

We next asked whether, within the populations of EBV-exposed cells, the relative distributions of isotype expression among EBV-infected and non-EBV-infected cells were similar. Populations of EBV-infected cells showed a reduced frequency of IgM-positive memory B cells compared to that in populations of non-EBV-infected cells ($P = 0.003$). This finding again suggested that, among memory B cells, EBV preferentially infects cells expressing IgA or IgG, i.e., memory B cells after class switching. These observations are consistent with

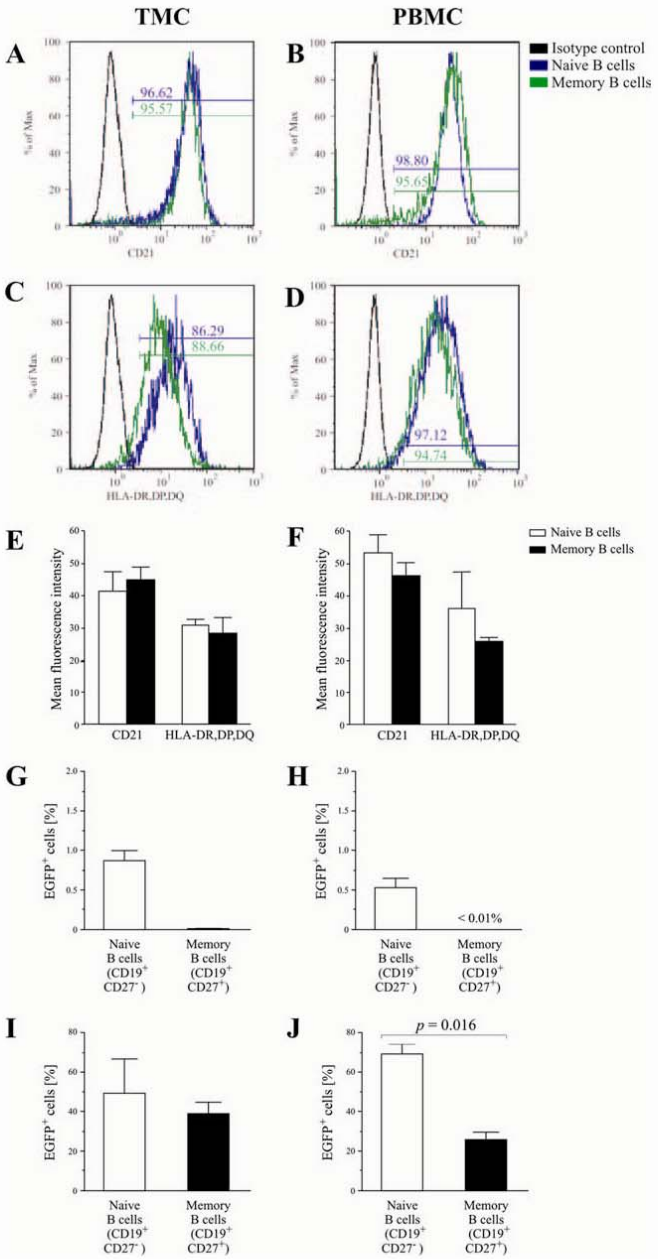

FIG. 4. B-cell subsets from tonsils, but not those from peripheral blood, are equally susceptible to EBV infection. (A to D) Numbers of naïve and memory B cells expressing the EBV receptors CD21 and HLA-DR. Cells were stained with antibodies against CD19, CD27, CD21, and HLA-DR, HLA-DP, and HLA-DQ. Flow cytometry plots are gated for CD19-expressing B cells and CD27-negative naïve or CD27-positive memory B cells. (E and F) Mean fluorescence intensities for CD21 and HLA-DR, HLA-DP, and HLA-DQ on naïve and memory B cells from tonsils and peripheral blood. (G and H) Hardly any infected memory B cells were detected among TMC (G) or PBMC (H) subjected to conven-

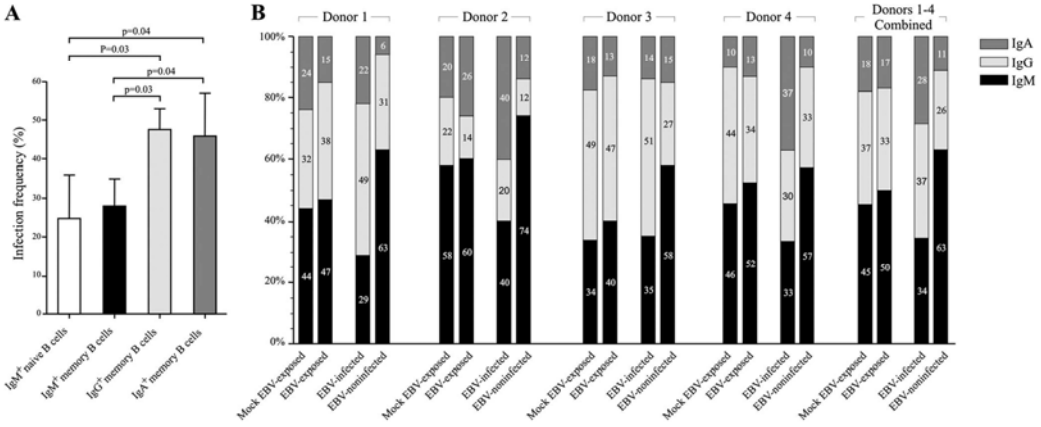

FIG. 5. Class switch recombination renders tonsillar memory B cells more susceptible than naïve B cells to EBV infection. (A) Infection frequencies for isolated IgM-positive and IgM-negative tonsillar memory B cells. Memory B-cell populations that did not undergo class switch recombination (IgM^+) showed significantly fewer infected cells 48 h postinoculation than their class-switched counterparts (IgG^+ and IgA^+). (B) Immunoglobulin isotype distribution among ex vivo EBV-infected tonsillar memory B cells 48 h postinoculation. The $EGFP^+$ (EBV-infected) population of the infected cells showed fewer IgM-positive cells than the corresponding $EGFP^-$ (uninfected) population. The whole population of infected cells showed no significant change in immunoglobulin composition compared to that of mock-infected controls. Results shown are from TMC from four donors and are expressed as means ± SEM. *P* values were calculated by paired Student's *t* test.

the findings in a recent report showing that EBV is preferentially excluded from the IgM-expressing memory B cells in vivo (36).

Tonsillar memory B cells are transformed by EBV at lower frequencies than tonsillar naïve B cells. Tonsillar memory B cells and naïve B cells showed similar EBV mRNA expression patterns after ex vivo infection; nevertheless, to test if their EBV-associated growth transformation potentials were identical, we infected whole populations of TMC, in addition to isolated tonsillar naïve and memory B cells, with B95.8 by spinoculation. We used wild-type B95.8 EBV in all transformation assays to exclude bias from the absence of LMP2 in the recombinant virus (B95.8EBfaV-GFP). The cells were cultured on an autologous feeder layer for 4 weeks to assess the transformation of EBV-infected B cells. Whole TMC populations and isolated tonsillar naïve B cells and tonsillar memory B cells infected with EBV showed the typical formation of cellular aggregates 48 h after spinoculation (data not shown), and the number of aggregates and their sizes steadily increased over the following 4 weeks (Fig. 6A to C) (29, 35). Conventional inoculation of TMC resulted in a transformation efficiency of around 60% when starting with 200 cells per well. In contrast, the spinoculation of a whole TMC population and isolated naïve B cells and memory B cells increased the transformation efficiency to more than 90% (Fig. 6B to D). Nevertheless, comparing the initial frequencies of infection of TMC that we observed using conventional inoculation or spinoculation with the transformation efficiencies suggested that using the conventional transformation assay (35) led to a state of initial saturation with EBV-infected B cells, which may lead to underestimation of the transformation efficiency. Thus, we performed a modified transformation assay, adding a limiting dilution of EBV-infected cells to a fixed number of B-cell-depleted autologous feeder cells. The transformation efficiency for naïve B cells was significantly higher than the one for memory B cells when cells were seeded at densities of 10 to 100 cells per well (Fig. 6E). Thus, memory B cells are transformed by EBV with a lower efficiency than naïve B cells. The micrographs in Fig. 6 show naïve and memory B cells at a cell density optimized for growth transformation. As seen in Fig. 6E, with the use of more than 100 naïve or memory B cells per well for transformation, no difference in transformation efficiency compared to that obtained with 100 cells per well is detectable.

LCLs from memory B cells exhibit less apoptosis and slower growth than LCLs from naïve B cells. In vitro, naïve B cells exhibit more apoptosis than memory B cells (7). We hypothesized that a change in apoptotic behavior after EBV infection may explain the differences in growth transformation efficiencies between naïve and memory B cells. As a marker for apoptosis, we analyzed the cleavage of PARP by Western blotting

tional EBV inoculation, which led to a very low rate of infection of the $CD19^+$ B cells. (I and J) Spinoculation led to a significant rise in the overall infection rate of the B-cell population. Among TMC, the proportions of naïve ($CD19^+$ $CD27^-$) and memory ($CD19^+$ $CD27^+$) B cells infected with EBV were similar (I), but among PBMC, the proportion of memory cells infected with EBV was lower than that of naïve B cells infected with EBV (J). Data are expressed as means ± SD of results from 10 independent experiments (A to F) and 3 independent experiments (G to J). *P* values were calculated by paired Student's *t* test.

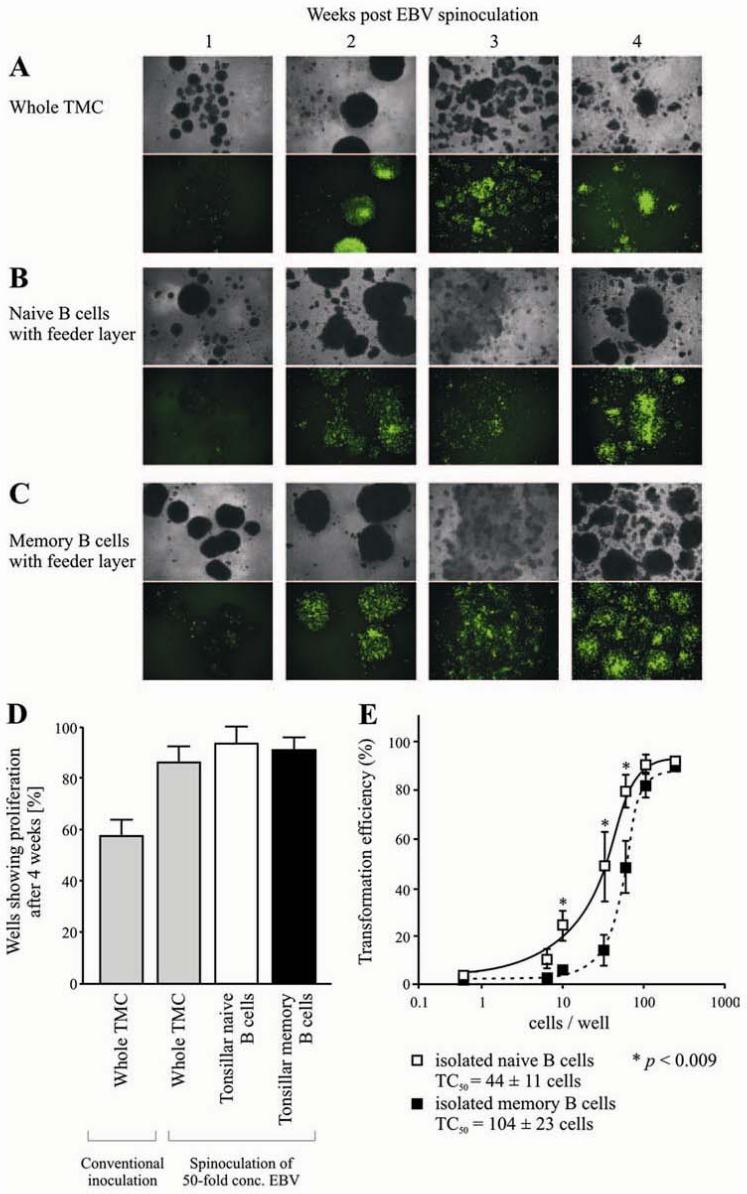

FIG. 6. Tonsillar memory B cells are less efficiently transformed by EBV than naïve B cells. (A to C) Whole TMC populations, naïve B cells, and memory B cells isolated from EBV-seronegative donors showed the classical picture of cell aggregate formation and the outgrowth of transformed cells 24 weeks after ex vivo infection using B95.8EBfaV-GFP. (D) Transformation efficiencies after conventional EBV inoculation and EBV spinoculation using the wild-type B95.8. TMC (2×10^5/well) infected with B95.8 were seeded into 96-well plates, and cells in wells showing proliferation after 4 weeks and B-cell levels above 98% were considered to be transformed. For the transformation of different B-cell subsets, samples of 250 naïve or memory B cells were spinoculated using B95.8, mixed with 2×10^5 B-cell-depleted TMC, and seeded into each well in 96-well plates. After 4 weeks, cells in wells showing proliferation and B-cell levels above 98% were considered to be transformed. conc., concentrated. (E) Transformation efficiencies of tonsillar naïve and memory B cells under conditions of limiting dilution. Naïve and memory tonsillar B cells were inoculated with B95.8 supernatants by spinoculation and were plated in 96-well plates together with an autologous feeder layer (2×10^5 cells/well). Cells in wells still showing growth and B-cell levels above 98% after 4 weeks were regarded to be transformed. Results are means ± SD from three independent experiments. *P* values were calculated by paired Student's *t* test. TC_{50}, 50% transformation concentration.

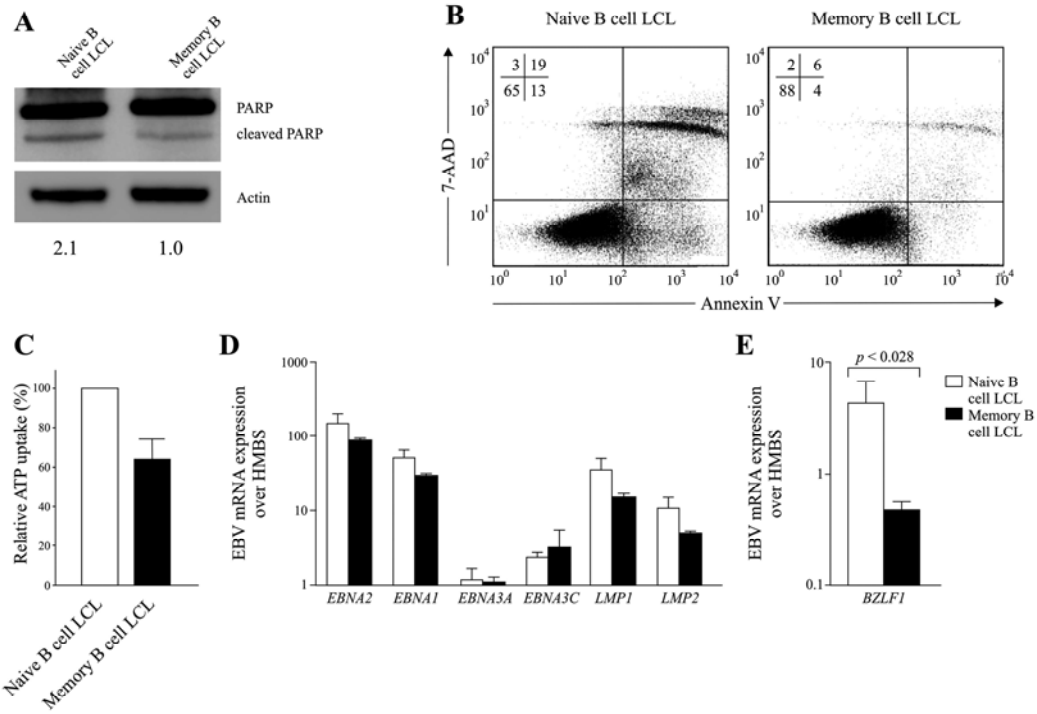

FIG. 7. Naïve and memory B cells retain their distinct apoptosis and proliferation characteristics after EBV-induced transformation. (A) Naïve and memory B cells show different apoptotic behaviors after EBV growth transformation. LCLs from naïve B cells are more susceptible to apoptosis than the autologous memory B cells, as assessed by Western blotting to detect the cleavage of PARP. Numbers below the blot indicate the level of PARP cleavage (*n*-fold) as normalized to actin and to PARP cleavage in memory B-cell LCLs. (B) LCLs from memory B cells show a lower proportion of cells undergoing apoptosis than LCLs from naïve B cells as measured by staining with Annexin V and 7-AAD. (C) LCLs from memory B cells as assessed by ATP uptake. (D) LCLs from naïve B cells and those from memory B cells express mRNA of the same set of EBV latency genes at equal levels, as determined by real-time PCR analysis. (E) EBV-transformed naïve B cells show a higher frequency of cells undergoing lytic EBV reactivation than EBV-transformed memory B cells, as measured by the mRNA expression of *BZLF1*. HMBS, hydroxymethylbilane synthase mRNA.

(10). We also determined the numbers of naïve and memory B cells undergoing spontaneous apoptosis after EBV-associated growth transformation into LCLs by staining with Annexin V and 7-amino-actinomycin D (7-AAD). LCLs from naïve B cells showed twofold-higher levels of PARP cleavage than LCLs from memory B cells (Fig. 7A). The frequency of apoptosis in LCLs from naïve B cells was around threefold higher than that in LCLs from memory B cells, i.e., 13.1% ± 6.5% compared to 4.2% ± 2.8% (P = 0.038) (Fig. 7B), when determined by staining with Annexin V and 7-AAD. Thus, after EBV transformation, naïve B cells showed significantly more apoptosis than did memory B cells. Therefore, these results alone could not explain the somewhat lower transformation efficiency of memory B cells than of naïve B cells and led us to hypothesize that the difference in transformation efficiency was linked to unequal proliferation rates.

We assessed the proliferation characteristics of naïve and memory B cells before and after EBV-induced growth transformation into LCLs by measuring the ATP uptake by the cells over 24 h. ATP uptake in newly isolated memory B cells was 47% ± 15% that in their naïve counterparts, implying that memory B cells grow more slowly (data not shown). EBV-transformed memory B cells showed 64% ± 9% of the ATP uptake shown by EBV-transformed naïve B cells (Fig. 7C). These results indicate that naïve and memory B cells retain their distinct apoptotic and proliferation behaviors following EBV transformation. That is, EBV-transformed memory B cells exhibit a lower frequency of apoptosis and proliferate more slowly than EBV-transformed naïve B cells.

Next, we asked whether the observed differences in proliferation were due to unequal EBV mRNA expression levels. LCLs obtained after the infection of naïve or memory B cells showed similar patterns of mRNA expression of EBV latent genes involved in B-cell transformation, with expression levels in naïve cells being slightly higher (Fig. 7D). By contrast, levels of *BZLF1* mRNA in LCLs from memory B

cells were significantly lower than those in LCLs from naïve B cells (Fig. 7E).

Compared to EBV-transformed naïve B cells, EBV-transformed memory B cells exhibit reduced apoptosis and lytic EBV gene expression. However, we believe that the slower proliferation of EBV-transformed memory B cells likely explains the lower EBV transformation efficiency of these cells in the dilution series experiments.

DISCUSSION

In this work, we aimed to assess the efficiencies of infection of distinct B-cell subsets from different lymphoid compartments and to compare the transformation behaviors of B-cell subsets infected ex vivo with EBV. Indeed, we found that tonsillar naïve and memory B cells were equally susceptible to ex vivo EBV infection but that peripheral blood memory B cells were less susceptible than naïve B cells. These data indicate that the tissue origin of memory B cells impacts the susceptibility to EBV infection. Moreover, among tonsillar memory B cells, the subset expressing IgM was less susceptible to EBV infection than the subsets expressing IgA or IgG, suggesting that class switching also impacts the susceptibility to EBV infection. Finally, even though tonsillar naïve and memory B cells displayed very similar patterns of EBV gene expression that is crucial to establish B-cell transformation, memory B cells showed significantly lower EBV-associated transformation efficiency than naïve B cells. This finding may be due to the inherently higher proliferation rates of naïve B cells than of memory B cells. Our findings, thus, reveal that the susceptibilities of B-cell subsets to EBV infection and their transformation capacities are not uniform and suggest that EBV exploits the status of differentiation and the compartment of origin of B cells to optimize the establishment of latency in the host.

Conventional protocols for EBV infection inoculate target cells with supernatant from EBV-positive cell lines that have been driven into lytic infection by mitogens or by the cross-linking of the B-cell receptor (9, 27). Such inoculation results in EBV infection of less than 2% of primary B cells (40). This low proportion hampers the clear dissection of what subsets of B cells are susceptible to EBV (39). To investigate the susceptibilities of distinct B-cell subsets, we needed to reach a higher infectivity rate. By using spinoculation with concentrated EBV, we increased the proportion of $CD19^+$ B cells infected with EBV to more than 50% at 48 h after inoculation. The entry process of EBV was almost completely inhibited when antibodies against the EBV envelope gp350/220 and gp42 or the B-cell receptors CD21 and HLA class II were added before spinoculation. This result clearly indicates that the increased infection frequency resulted from specific virus entry through the known EBV receptors and not from an unspecific uptake. Thus, the ex vivo EBV infection of B cells by spinoculation likely mimics the natural B-cell infection pathway and thus enabled us to study EBV infection in B-cell subsets from primary cells. Importantly, by using primary cells exclusively from EBV-negative individuals, we excluded bias from preexisting EBV-specific immunity or the necessity to apply immunosuppressive drugs in vitro in transformation experiments.

Indeed, using this optimized infection protocol, we found that memory B cells isolated from tonsils are susceptible to ex vivo EBV infection at frequencies similar to those of their naïve counterparts. This observation is in agreement with our finding of similar densities of the receptors for EBV (i.e., CD21 and HLA-DR) on tonsillar naïve and memory B cells, as defined by the expression of CD27. In contrast, among PBMC, the proportion of infected memory B cells corresponded to only one-third of that of infected naïve B cells (Fig. 2). Strikingly, the densities of receptors CD21 and HLA-DR on naïve and memory B cells among PBMC were similar. This observation of a tissue origin-dependent susceptibility rate of memory B cells was unexpected. We speculate that the priming of naïve B cells at different anatomic sites results in the distinct susceptibility of memory B cells to EBV infection and that EBV succeeds in optimally exploiting unknown cellular factors present exclusively in tonsillar memory B cells. Notably, the tonsils are the portal of entry for EBV. Our finding that naïve B cells from peripheral blood were susceptible to EBV ex vivo contrasts with the absence of EBV-infected naïve B cells in peripheral blood in vivo (17). Likely explanations for this discrepancy are that naïve B cells infected at the portal of entry for EBV are too short-lived to gain access to the circulation as long as they do not differentiate into long-lived memory B cells and that EBV virions or productively infected memory B cells are hardly found in the peripheral blood (2).

B cells expressing CD27 correspond to functional memory B cells, i.e., B cells having completed antigen-driven selection and differentiation and thus exiting or having left germinal centers (41). Our data, which show that EBV infects $CD27^+$ tonsillar B cells and $CD27^-$ tonsillar B cells with equal efficiencies, suggest that the degree of susceptibility to EBV infection is retained in B cells exiting the germinal center. We propose that functional memory B cells represent one direct target of EBV at its natural portal of entry in vivo. The infection of memory B cells in addition to naïve B cells at the portal of entry would provide EBV with an additional important biological advantage to increase the pool of infected B cells and thus secure persistence in the host. The targeting of functional memory B cells by EBV, either directly or indirectly via the infection of naïve B cells subsequently driven through a germinal-center reaction by the virus (37), holds the advantage that the EBV-infected B cell is subjected to proliferation and differentiation signals from cognate antigens when homing back to the tonsils. Tonsillar memory B cells are certainly more frequently exposed to antigens than, e.g., memory B cells from lymph nodes draining normally sterile body tissues. This scenario in turn would ensure that latent EBV is handed over to progeny cells. In addition, following the differentiation of the EBV-infected memory B cells into plasma cells, which represent the site of lytic replication (25), EBV could gain access to other susceptible B cells or epithelial cells and, via saliva, to susceptible new hosts.

Our data clearly demonstrate that among $CD27^+$ memory B cells, those expressing IgA or IgG are significantly more susceptible to EBV infection than those expressing IgM. This fact suggests that if EBV uses tonsillar memory B cells as a direct target, the virus preferentially accesses isotype-switched cells. In agreement with our observations, the vast majority of EBV DNA in peripheral blood cells from EBV carriers has been detected in surface-IgA-positive B cells (12). Moreover, recent work on peripheral blood B cells isolated from patients with

infectious mononucleosis found that EBV-infected cells were preferentially excluded from the IgM isotype in the $CD27^+$ memory B-cell pool (36). Based on this finding and on the presence of more frequent somatic hypermutations in EBV-infected than in non-EBV-infected $CD27^+$ B cells, the authors of the study concluded that EBV impacts the infected cells themselves by promoting their germinal-center reaction with isotype switching (36). Thus, it appears that EBV infects B cells at different differentiation stages.

A remarkable finding of this work is that tonsillar memory B cells manifest significantly lower EBV-induced transformation efficiency than their naïve counterparts. The infection of B cells with EBV in vitro is well-known to result in the expression of the so-called latency III viral gene expression program. This program is characterized by the expression of six nuclear antigens (EBNA1 to EBNA6), LMP1, and LMP2 (33), which are required for establishing EBV latency and EBV-induced growth transformation and immortalization (13). Both tonsillar B-cell subsets expressed mRNA of *EBNA2*, the master regulator of latency, and mRNAs of *EBNA1*, *LMP1* and *LMP2*, and *EBNA3A* and *EBNA3C*, which are also important for EBV growth transformation (1) following infection with EBV in vitro. Importantly, the mRNA expression levels of the latency genes involved in cellular transformation were similar in naïve and memory B cells, suggesting what would let us expect similar EBV-induced transformation efficiencies for the two B-cell subsets. The higher apoptosis frequency and higher mRNA expression levels of *BZLF1* in transformed naïve than in memory B cells, indicating greater initiation of spontaneous EBV lytic infection in LCLs from naïve B cells, would rather suggest a lower transformation rate of naïve B cells, which is, however, not the case. Therefore, qualitative and quantitative EBV gene mRNA expression patterns alone do not allow firm conclusions on the host-pathogen interactions.

Another explanation for the distinct transformation capacities of B-cell subsets may be a difference in cell proliferation rates. In fact, we found that the lower transformation efficiency of memory B cells was paralleled by their reduced rate of proliferation compared to that of naïve B cells. Importantly, the proliferation rates of these two B-cell subsets differed before and after EBV-induced transformation. This result suggests that infection with EBV does not significantly alter the proliferation characteristics associated with B-cell differentiation.

In conclusion, our data demonstrate that spinoculation enhances EBV infection without bypassing the requirements for the normal binding of EBV to or the entry of EBV into B cells and, therefore, offers a highly valuable in vitro tool to study EBV infection, host-pathogen interactions, and pathogenesis. This tool allowed the first-time quantification of striking differences in susceptibility to EBV infection and EBV-induced transformation efficiency between B-cell subsets. These differences were dependent on the tissues of origin and the differentiation statuses of the cells, suggesting that EBV exploits the characteristics of B-cell subsets to optimize the establishment of latent infection.

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Integrin expression determines the susceptibility of memory B cell subpopulations to EBV infection

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Abstract

The Epstein-Barr virus uses the tonsils as portal of entry to establish life-long persistence within the memory B cell pool. Even though it is generally believed that transmission of EBV occurs via saliva, the exact mechanisms of how EBV gains access to the memory B cell pool is still unclear. In this work, we show how EBV selectively infects only memory B cells that were generated within the nasopharynx-associated lymphatic tissue *ex vivo*, with memory B cells from other lymphatic organs being less susceptible to infection. Furthermore, we demonstrate that memory B cells that were selected via affinity maturation within the nasopharynx-associated lymphatic tissue are susceptible to EBV infection, as they express high levels of $\alpha_5\beta_1$ Integrin, which, upon binding of EBV, delivers signals ultimately leading to enhanced EBV entry via phosphorylation of FAK, c-Src, PI3K p85α, cofilin and actin depolymerization. This preferential infection of NALT memory B cells in turn leads to the exclusive transformation of memory B cells with specificity against respiratory pathogens, thereby explaining, why antigen-specific cell lines reacting to non-respiratory antigen could not be generated in the past.

Introduction

The Epstein-Barr (EBV) virus is a ubiquitous human γ-herpesvirus associated with Burkitt's lymphoma and post-transplant lymphoproliferative disease (Rickinson and Kieff, 2001). Even though primary infection with EBV during childhood is usually asymptomatic, adults may develop symptoms of infectious mononucleosis (IM) (Cohen, 2000). The acute phase of EBV infection, whether it is accompanied by IM or not leads to the establishment of life-long persistence of the virus within the B cell pool of the host (Babcock et al., 1998; Miyashita et al., 1997). The portal of entry of EBV is the lymphatic tissue of the Waldeyer's ring, where EBV enters the tonsillar B cell-rich areas to establish latent infection (Berger et al., 2007). During the course of infection, EBV is able to infect B cells at all maturation stages, including naïve (Joseph et al., 2000; Thorley-Lawson and Babcock, 1999; Thorley-Lawson and Gross, 2004), germinal center (GC) (Bechtel et al., 2005; Kuppers, 2005; Mancao et al., 2005) or memory B cells (Dorner et al., 2008; Ehlin-Henriksson et al., 2003; Kurth et al., 2000) within the tonsil. Depending on the differentiation of the B cells at the time-point of infection, the expression of latent EBV genes varies from nearly all EBV latent genes being expressed in naïve B cells to a very restricted expression in memory B cells (Babcock et al., 2000). After the EBV-infected memory B cells exit the lymphatic tissue of the tonsil and enter the peripheral circulation, EBV evades immune recognition by completely shutting down latent gene expression in resting memory B cells (Babcock and Thorley-Lawson, 2000; Miyashita et al., 1997). Only after the EBV-infected memory B cells home back to the tonsils and are activated through the recognition of an apt antigen, EBV reactivates from latency during the process of plasma cell differentiation (Laichalk and Thorley-Lawson, 2005).

EBV has been used in the past to generate Lymphoblastoid cell lines secreting antibodies specific for respiratory pathogens such as influenza or SARS coronavirus (Lanzavecchia et al., 2006; Lanzavecchia et al., 2007; Simmons et al., 2007; Traggiai et al., 2004; Zhu et al.,

2007). Recently, we could demonstrate that EBV preferentially infects memory B cells from tonsils, but not those from peripheral blood (Dorner et al., 2008). Memory B cells present within the peripheral blood exhibit specificities for both, respiratory and non-respiratory pathogens, thus resembling a mixture of memory B cells originating from the NALT and other lymphatic tissues (Brandtzaeg et al., 1999). In contrast, memory B cells from the tonsils are mainly generated by affinity-maturation to respiratory pathogens.

Here we show that memory B cells originating from the NALT, the portal of entry of EBV are susceptible to EBV infection and growth transformation, thus giving raise to antibody-producing LCLs with a specificity for respiratory pathogens. In contrast, memory B cells from other lymphatic tissue are not susceptible to EBV infection and transformation thereby explaining the absence of antibody production against non-respiratory pathogens. Furthermore, we identified the reason for this difference between memory B cells from distinct lymphatic tissues to be the presence or absence of $\alpha_5\beta_1$ integrin. Absence of $\alpha_5\beta_1$ integrin on non-NALT memory B cells leads to decreased EBV infection and transformation. Even though described solely as an attachment factor for EBV on epithelial cells, we present data showing that also integrin-mediated signalling is essential for efficient infection of memory B cells with EBV.

Results

Memory B cells originating from NALT are more susceptible to EBV infection and transformation than their counterparts from other lymphatic organs.

Memory B cells are the main reservoir for EBV persistence *in vivo* and they are also susceptible to direct infection with EBV *in vitro*, even though there appear to be tissue-specific differences (Babcock et al., 1998; Dorner et al., 2008). To investigate these differences in detail, we performed infection assays using spinoculation of high-titer EBV as previously described (Dorner et al., 2008).

Memory B cells from tonsils, which are part of the Waldeyer's ring forming the NALT, show the highest susceptibility to EBV infection with 75.8 ± 4.9 % of infected cells 24 hours post inoculation (Fig 1A). In contrast, memory B cells from peripheral blood, which is a mixture of memory B cells from all lymphatic compartments, could only be infected in 30.3 ± 13.6 % of cells. To dissect the distinct memory B cell subpopulations originating from NALT and non-NALT, we exploited the presence of L-selectin (CD62L) on memory B cells from NALT to distinguish between NALT and non-NALT-originating memory B cells (Brandtzaeg and Johansen, 2005). Tonsils, in which the NALT-originating memory B cells are generated, contain 81.6 ± 6.7 % $CD19^+CD27^+CD62L^+$ NALT memory B cells indicating that only 19 % of the memory B cells have homed to the tonsil from different lymphatic organs (Fig. 1B). In contrast, only 21.5 ± 6.9 % of the $CD19^+CD27^+$ memory B cells express CD62L with over 79 % of the memory B cells originating in non-NALT.

Infection of memory B cells from tonsils and peripheral blood yielded a similar pattern of EBV infection. $CD19^+CD27^+CD62L^+$ memory B cells from tonsils and peripheral blood showed a higher infection frequency (75.8 ± 5.0 % and 67.8 ± 7.9 %, respectively) compared to $CD19^+CD27^+CD62L^-$ memory B cells, which showed lower infection frequencies in tonsils

and peripheral blood (40.0 ± 7.0 % and 16.0 ± 4.7 %, respectively). EBV infection did not alter the expression pattern of CD62L (data not shown).

EBV-associated transformation efficiency is higher in NALT memory B cells, enabling the generation of antibody-producing LCL against respiratory antigen.

Efficient infection of memory B cells by EBV is a prerequisite to efficient generation of antibody-producing LCL. As the initial infection of $CD62L^-$ memory B cells originating from non-NALT is hampered, the transformation efficiency of these cells should also be lower compared to $CD62L^+$ memory B cells from NALT. To address this question, we performed transformation assays using limiting dilutions of EBV-infected memory B cells from tonsils (majority $CD62L^+$) and from peripheral blood (majority $CD62L^-$) on autologous B cell-depleted feeder cells (Fig. 2A). Memory B cells from tonsils exhibit a three-fold higher transformation efficiency compared to those from peripheral blood. Thus, the question arose, whether the low infection frequency leading to the low transformation efficiency of non-NALT-originating memory B cells is also reflected by the antibodies produced from the associated LCLs. Vaccinations, which are routinely applied via intramuscular injection lead to an initial round of affinity maturation of B cells within the lymph nodes rather than the lymphatic tissue of the nasopharynx. We therefore chose tetanus toxoid (TT) as a model antigen, identifying the lymph-node-selected memory B cells within the peripheral blood. Detection of TT-specific memory B cells was performed using an enzyme-linked immunosorbent spot (EliSPOT) assay (Suzuki and Tagami, 2005) and identified high numbers of TT-specific memory B cells six days post immunization (Fig. 2B). Following EBV-associated transformation of the memory B cells from peripheral blood, antigen-specificity against TT could not be detected. To further validate our data, we performed

ELISA detecting anti-TT antibodies verifying the results observed in the EliSPOT assays (Fig. 2B). Controls for these assays included donors that did not receive any TT vaccination.

In contrast to immunizations, wild-type infection with respiratory pathogens that do not lead to systemic viremia are restricted to generating antigen-specific memory B cells within the lymphatic tissue of the Waldeyer's ring. Thus, we chose measles wild-type infection as a potential source of NALT-originating measles-specific memory B cells from peripheral blood which were detected with a measles virus IgG ELISA. In healthy donors without booster vaccination, only background antibody titers were detectable, whereas the anti-measles IgG titer in booster vaccinated and wild-type measles virus infected individuals were greatly increased. Booster vaccination with measles virus vaccine should, as observed for TT, primarily lead to affinity maturation of B cells within axillary lymph nodes. Following EBV transformation of B cells of measles virus boosted donors, anti-measles IgG production was completely absent irrespective of the initial anti-measles IgG titer after immunization. In contrast, EBV transformed B cells from wild-type measles infected donors showed constant levels of antibody production indicating that affinity maturation in the NALT is mandatory for EBV to infect the antigen-specific memory B cell.

The EBV-attachment factor $\alpha_5\beta_1$ integrin is highly expressed on memory B cells from NALT.

Next, we were interested in identifying potential candidates explaining the higher infection susceptibility of memory B cells from NALT compared to those of other lymphatic organs. Recently, it has been described that the integrin receptor family, especially the α_3, α_V, and β_1 subunits, might play an important role in EBV attachment to epithelial cells (Xiao et al., 2008; Xiao et al., 2007). This binding is facilitated via a novel EBV glycoprotein, BMRF2 (Xiao et al., 2007).

To identify, whether the differential susceptibility of memory B cells from NALT and non-NALT might be due to dissimilar $\alpha_5\beta_1$ integrin expression levels, we isolated memory B cells from tonsils and peripheral blood and subjected them to immunofluorescence staining and flow cytometry for $\alpha_5\beta_1$ integrin. Immunofluorescence shows a weaker expression of $\alpha_5\beta_1$ integrin on memory B cells from peripheral blood compared to tonsils even though all cells express β_1 integrin at low levels (Fig. 3A, B). Quantification of the β_1 expression levels validated the findings observed in immunofluorescence with 95.1 ± 4.4 % and 83.6 ± 3.1 % of β_1-positive memory B cells from tonsils and peripheral blood, respectively (Fig. 3C). Analysis of the mean fluorescence intensity of $\alpha_5\beta_1$ integrin on these cells revealed, that memory B cells from tonsils express 8- to 10-fold more β_1 integrin compared to those from peripheral blood (Fig. 3D).

EBV binds to $\alpha_5\beta_1^{high}$ memory B cells, thereby enhancing the infection susceptibility of NALT-originating memory B cells.

Next, we addressed the question, whether EBV uses β_1 integrin to attach to NALT-originating memory B cell as it does with epithelial cells. The B-cellular infection with EBV has been extensively studied, identifying two cellular receptors important for EBV binding and entry into B cells (Miller and Hutt-Fletcher, 1992; Spear and Longnecker, 2003; Speck et al., 2000). The main attachment factor for EBV on B cells is CD21 (CR2, C3R), which is a member of the complement receptor family. Binding of EBV to CD21 via the viral glycoprotein gp350/220 has been shown to induce receptor-mediated endocytosis. Within the endosomal compartment, a trimer of EBV glycoproteins consisting of gH, gL and gp42 binds to HLA class II. This interaction in turn triggers a pH-dependent membrane fusion of EBV with the endosomal membrane and allows nuclear transport of the capsid.

To test, whether EBV uses $\alpha_5\beta_1$ integrins on NALT memory B cells for attachment, we performed blocking experiments using either antibodies blocking EBV binding to $\alpha_5\beta_1$ integrins or soluble recombinant β_1 integrin to compete with EBV binding to cellular β_1 integrin. When using either anti- $\alpha_5\beta_1$ blocking antibodies alone or in combination with anti-CD21 blocking antibodies, a dose-dependent reduction of the EBV infection frequency could be observed in tonsillar memory B cells, but not in peripheral blood memory B cells (Fig. 4A, B). Blocking of CD21 alone or blocking of HLA class II molecules was also included as positive control and showed a significant reduction of EBV infection frequency for both CD21 and HLA class II.

Additionally, recombinant soluble β_1 integrin, either with or without additional inhibition of the EBV glycoprotein gp350/220, showed the same dose-dependent inhibition of EBV infection of memory B cells from tonsils, but not from peripheral blood (Fig. 4C, D). As positive control, we also included blocking of gp350/220 alone or in combination with limiting dilutions of anti-gp42 to also block EBV penetration of the endosomal membrane.

Thereby, we could show by either blocking cellular β_1 integrin or by competing the β_1 integrin with recombinant protein that EBV indeed uses them as attachment factors not only to epithelial cells, but also to NALT-originating memory B cells.

Integrin signalling is facilitating EBV entry into NALT-originating memory B cells.

Even though the requirement of EBV binding to epithelial cells for EBV infection could be shown, the participation of signalling events downstream of $\alpha_5\beta_1$ integrin initiated through EBV binding and their contribution to EBV entry have not been elucidated. We therefore dissected the integrin signalling pathway following EBV binding (Hehlgans et al., 2007; Sixt et al., 2006). One of the initial steps in integrin signalling involves activation of focal adhesion kinase $_{397}$Tyr phosphorylation followed by downstream signalling through PI3K, c-

Src kinases and cofilin to ultimately activate the depolymerization of the actin cytoskeleton (Hehlgans et al., 2007; Huveneers et al., 2007; Rose et al., 2007; Shi and Simon, 2006; Yee et al., 2008). To investigate, whether EBV itself activates integrin signalling thereby enhancing infection efficiency, we (i) longitudinally followed the phosphorylation status of FAK, PI3K and cofilin upon binding of EBV and (ii) treated isolated memory B cells from tonsils with inhibitors of *c*-Src, PI_3 kinases or FAK activation prior to infection with EBV. Binding of EBV to integrins leads to a rapid upregulation of pFAK within 20 minutes, which is followed by phosphorylation of c-Src, PI3K and cofilin (Figure 5A). Furthermore, protrusions in the actin cytoskeleton are readily detectable in memory B cells when binding of EBV occurs (Figure 5B). Inhibition of signalling molecules downstream of integrins led to a marked reduction of EBV infection frequency compared to mock treated cells (Fig. 5C,D). In addition, we also induced actin depolymerization, which is normally activated via the integrin signalling pathway. This showed a significant increase of the overall infection frequency of memory B cells with all cells being susceptible to EBV infection.

Discussion

In this work, we aimed at assessing the susceptibility of memory B cells from distinct secondary lymphoid organs to EBV infection and transformation and to identify cellular factors influencing EBV entry into these cells. Indeed, we found that memory B cells from the nasopharynx-associated lymphatic tissue are more susceptible to EBV infection than their counterparts originating from other lymphatic tissue. We could show this by distinguishing NALT-originating $CD62L^+$ memory B cells from non-NALT-originating $CD62L^-$ memory B cells in the tonsils and peripheral blood before and after infection with EBV. This difference of NALT- and non-NALT memory B cells in susceptibility to EBV infection consecutively manifested in the transformation efficiency of memory B cells from tonsils and peripheral blood. The two-fold lower infection frequency of peripheral blood memory B cells compared to tonsillar memory B cells led to a 2- to 5-fold reduced transformation efficiency of peripheral blood memory B cells. This furthermore explains, why in the past, only memory B cells selected against respiratory antigen could be efficiently transformed with EBV for the production of monoclonal antibodies. This could also been shown by measuring the antigen-specificity of the EBV-transformed peripheral blood memory B cells. Even though the transformation efficiency at the employed cell density was above 50%, no antigen-specificity for tetanus toxoid could be detected. As this antigen was not delivered via the NALT, memory B cells were generated against TT mainly within the axillary lymph nodes but not within the tonsils and therefore were part of the $CD62L^-$ memory B cell pool that is not infected with EBV efficiently.

In contrast, memory B cells from donors that recently underwent wild-type measles virus infection, which mainly affects the respiratory tract, could be shown to retain their antigen-specificity throughout the process of EBV transformation. Antigen-specificity was determined

using EliSPOT and Elisa using fresh blood from TT-vaccinated or measles virus-infected or healthy individuals as positive and negative controls, respectively.

The mechanisms by which the susceptibility of cells to EBV infection is regulated have only been described for epithelial cells and B cells, as epithelial cells do not express the known B-cellular receptors, CD21 and HLA class II, crucial for EBV entry. As memory B cells from distict lymphatic tissue express equal amounts of CD21 and HLA class II, respectively, the observed differences in infection susceptibilities of memory B cells from different lymphatic sites cannot be explained by differential expression levels of CD21 and/or HLA class II. Recently, it has been described that the family of integrin transmembrane rececptors may play a role in the attachment of EBV to epithelial cells. We therefore chose the most promising candidate, the $\alpha_5\beta_1$ subunit of integrins to further investigate, whether this receptor is also expressed on memory B cells. Indeed, we found that, even though all memory B cells expressed $\alpha_5\beta_1$ integrin irrespective of the origin, memory B cells from tonsils expressed $\alpha_5\beta_1$ integrin at 10-fold higher levels compared to their counterparts from peripheral blood. As blocking of $\alpha_5\beta_1$ integrin by using either blocking antibodies or the recombinant extracellular domain showed a dependence of EBV entry into memory B cells, we show for the first time, that the EBV glycoprotein BMRF2 - $\alpha_5\beta_1$ integrin interaction is required and responsible for the distinct infection susceptibilities of memory B cells.

The binding of EBV BMRF2 to $\alpha_5\beta_1$ integrin induces signalling through crosslinking of $\alpha_5\beta_1$ leading to the activation of the downstream target focal adhesion kinase, which in turn activates PI_3 kinases and *c*-Src. This in turn leads to activation of actin depolymerization which we could show to have a benefitial effect on EBV internalization by Cytochalasin D activation of actin depolymerization.

Our data clearly demonstrate that EBV itself, via its glycoprotein BMRF2, determines the specificity for infection of memory B cells from different lymphatic tissue, preferentially

infecting memory B cells originating in the tonsil, the portal of entry of EBV. The inefficient infection of memory B cells from other lymphatic tissue than the NALT is furthermore reflected by a 2- to 5-fold reduced transformation capacity and the inability of memory B cells specific for non-respiratory antigen to undergo EBV-associated transformation. These findings also potentially shed light on why EBV-associated Burkitt's lymphoma and Oral hairy leukoplakia are most frequently found to originate from regions of the NALT. If the infection frequency of NALT memory B cells is two fold higher than that of non-NALT memory B cells, these cells are also more likely to develop oncogenic mutations in response to the EBV infection.

Materials & Methods

Cell culture and virus

All cells were maintained in RPMI1640 supplemented with 10% FBS, 1% L-Glutamine, 1% Penicillin/Streptomycin, 1x Sodium pyruvate and 1x non-essential amino acids. B27 cells containing a BMRF2 knock-out EBV were kindly provided by Sharif Tugizov (…..). For virus production, the B95.8EBfaV-GFP cell line or the B27 BMRF2 ko cell line were used and cells were cultivated for 4 days at a cell density of 10^6 cells/mL in the presence of 50 ng/mL 12-O-tetradecanoylphorbol 13-acetate (TPA). Following centrifugation of cells for 300xg for 10 minutes and filtration of the supernatant through a 0.45µm membrane filter, supernatants were centrifuged for 2 hours at 17.000 rpm at 4°C to pellet EBV. The resulting pellet was then resuspended in TB-Sal buffer to obtain a concentration of 10 mg total protein per mL. Inactivation of EBV was performed either by heat-inactivation at 65°C for 1 hour or by UV-irradiation (254 nm, 1500 J…..).

Antibodies and chemicals

The used antibodies against CD19, CD27, CD29 and CD62L were purchased from BD Biosciences. The tetanus toxoid antigen, the anti-measles virus serum and the anti-tetanus antibody were supplied from the National Institute of Biological Standards and Control. The Tetanus vaccination Tetanol® Pur was from Novartis and the measles vaccination M-M-RVaxPro® was from Sanofi-Pasteur.

The measles virus antigen was a previously described B cell receptor epitope peptide{Zvirbliene, 2007 #671} purchased from Eurogentec.

The function blocking antibody against $\alpha_5\beta_1$ integrin and EBV gp350/220 were from Abcam, the soluble recombinant β_1 integrin was from Thermo Scientific and the blocking antibodies against CD21 and HLA-DR,DP,DQ were purchased from Biolegend. The blocking antibody

against EBV gp42 (clone F2.1) was kindly provided by Dr. Hutt-Fletcher. The chemical inhibitors PP2, Wortmannin, Cytochalasin D and AG-82 were from Calbiochem and Ly-294002 was from Sigma-Aldrich. Antibodies detecting either pY397 FAK or total FAK were from BD Biosciences. Antibodies detecting pY416 c-Src, total c-Src, pY458 pI3K p85α, total PI3K p85α, pS3 cofilin, total cofilin and all HRP-conjugated secondary antibodies were from Cell Signalling (……).

Isolation of cells

Human mononuclear cells were isolated from tonsils obtained from EBV-seronegative donors undergoing routine tonsillectomy and from peripheral blood of EBV-seronegative donors. Tonsillar mononuclear cells were prepared as previously described. Briefly, tonsils were disintegrated using a scalpel and the cell suspension was passed through a 70 μm cell strainer. After washing the cells with PBS, mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation.

Mononuclear cells from peripheral blood were also isolated by Ficoll-Paque density gradient centrifugation.

Further separation of mononuclear cells into different lineages or differentiation stages was performed using magnetic bead isolation using the B cell isolation kit II and CD27 microbeads to isolate memory B cells.

Infection of cells

Infection of memory B cells with EBV was performed as described earlier with minor modifications. Briefly, 10% (v/v) of high-titer EBV in TB-Sal was added to the memory B cells (10^6 cells/mL) to obtain a final volume of 100 μL. The cells were then subjected to

spinoculation at 4°C at 800xg for 60 minutes. After washing the cells twice with ice-cold PBS, cells were resuspended in fresh RPMI1640.

Transformation assay

To determine the transformation efficiency, B cell-depleted mononuclear cells were added to 96-well plates as an autologous feeder layer at a cell density of 10^5 cells/well in a total volume of 80 µL. Isolated memory B cells from tonsils or peripheral blood were infected with EBV by spinoculation and added to the feeder layer at limiting dilutions from 1 to 10^4 cells resulting in a total volume of 100 µL. Half of the medium was changed every 3 days until transformation occurred after 4 weeks. Wells still showing proliferation and B cell counts above 95% were considered to be transformed. At least 96 wells were used in every replicate.

EliSPOT and Elisa

MultiScreen-HA Filter Plates with mixed cellulose ester membrane (Millipore) were prewet with 40 µL 35% ethanol. After washing the plate three times with MilliQ water, the plate was coated using either tetanus toxoid (10 µg/mL) or the measles virus B cell epitope peptide (100 µg/mL) in carbonate buffer pH 9.6 for 16 hours at 4°C. After washing the plates 4 times with PBS, they were blocked for 1 hour at 37°C using 3% BSA fraction V in PBS. Mononuclear cells, isolated 6-8 days post vaccination or EBV-transformed LCL were added to the plate at various concentrations between 10^2 and 10^6 cells/well in RPMI1640 and the plates were incubated at 37°C and 5% CO_2 for 6 hours. After washing away all cells first using 3% BSA fraction V in PBS + 0.05% Tween 20 followed by three consecutive washing steps with 3% BSA fraction V in PBS, horseradish-peroxidase (HRP)-conjugated mouse anti-human IgG (Fc) (ABD Serotec) was added to the plates at a dilution of 1:2000 in PBS + 3% BSA fraction V and plates were incubated at 18°C for 1 hour. Washing of the plates to remove unbound

antibody was performed three times with 3% BSA fraction V in PBS followed by the development step using TMB substrate (Mabtech) for 20 minutes at 18°C. To stop the development, the plates were washed extensively with MilliQ water and allowed to dry for 16 hours at 4°C. Spots were counted manually using a Binocular microscope.

Elisa detecting TT- or measles virus-specific IgG were performed on microtiter plates (Corning) using the same antigen concentration as used for EliSPOT. All washing steps are equal to the EliSPOT protocol. Coating was performed in carbonate buffer pH 9.6 at 4°C for 16 hours. Blocking was performed for 1 hour at 18°C using 3% BSA fraction V in PBS followed by incubation with HRP-conjugated anti-human IgG (Fc) and development with TMB substrate. A standard curve using anti-TT antibody or anti-measles serum was also included.

Inhibition of EBV infection

Inhibition of EBV entry receptors on memory B cells was performed by incubating isolated memory B cells with either anti-human CD21 (1:10), anti-human HLA-DR,DP,DQ (1:10) or various dilutions of anti-β_1 integrin for one hour at 4°C followed by spinoculation of the cells. Inhibition of EBV glycoproteins was performed by incubation of the EBV-containing supernatants with either anti-gp350/220 (1:10), various dilutions of anti-gp42 or soluble recombinant β_1 integrin for 1 hour at 4°C prior to spinoculation of memory B cells.

Inhibition of the downstream integrin signaling was performed by incubating the isolated memory B cells with either PP2 (10 μM), wortmannin (10 nM), Ly-294002 (10 μM), AG-82 (10 μM) or cytochalasin D (10 μM) for 30 minutes prior to spinoculation with EBV.

Western blotting

After washing the cells twice with ice-cold PBS, 5 x 10^6 cells were resuspended in 200 µL modified RIPA buffer containing 1% Igepal CA-630, 0.5% Sodium deoxycholate, 0.1% Brij35, 10 mM β-mercaptoethanol, 10 µg/ml PMSF, 5 µg/ml aprotinin, 0.1 mg/ml benzamidine, 1 µg/ml pepstatin A, 1 µg/ml leupeptin, 100 mM sodium orthovanadate, 1 mM Sodium fluoride and 15 µl/ml Triton X-100 in PBS. Antibodies used were added at a dilution of 1:1000 and were detected using SuperSignal Femto Western Blot detection system (Thermo Scientific…..)

Fluorescence microscopy

For the detection of actin rearrangements, isolated memory B cells were serum-starved for 6 hours before performing EBV binding reactions. At defined time-points, cells were washed with ice-cold PBS and were cytospun on microscopy slides. Fixation was performed for 20 minutes in 4% paraformaldehyde prior to permeabilization using 0.1% TritonX-100. Staining of actin was done using FITC Phalloidin and slides were ……

Flow Cytometry and Fluorescence Activated Cell Sorting

Flow cytometry was performed using a Cytomics FC 500 (Beckman Coulter)

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Figure legends

Figure 1. EBV preferentially infects CD62L$^+$ memory B cells from NALT.

(A) Infection frequency of memory B cells from tonsils, peripheral blood and lymph nodes. Results shown are EGFP$^+$ cells gated for CD19$^+$CD27$^+$ memory B cells. (B) CD62L expression on CD19$^+$CD27$^+$ memory B cells from tonsils, peripheral blood and lymph nodes. (C) CD62L expression on memory B cells from tonsils, peripheral blood and lymph nodes before and 24 hours after EBV infection. (D) Infection frequency of CD62L$^+$ and CD62L$^-$ memory B cells from tonsils, peripheral blood and lymph nodes. Results shown are mean ± SD from 4-8 replicates.

Figure 2. Transformation of memory B cells by EBV is only efficient in memory B cells specific for respiratory antigen.

(A) Transformation efficiency of tonsillar and peripheral blood memory B cells (TC_{50} transforming concentration 50). (B, C) Frequency of tetanus toxoid-specific memory B cells in peripheral blood of TT-naïve or TT-immunized donors before and after EBV transformation measured by (B) EliSPOT and (C) Elisa. (D) Frequency of measles virus-specific memory B cells in peripheral blood of MV-naïve, MV-immunized and MV-infected donors before and after EBV transformation measured by Elisa. Data shown are means ± SD of three biological replicates.

Figure 3. Integrin β_1 is preferentially expressed on memory B cells from NALT.

(A, B) Immunofluorescence of $\alpha_5\beta_1$ integrin expression on memory B cells from (A) tonsil, and (B) peripheral blood. (C-K) Detection of $\alpha_5\beta_1$ integrin on memory B cells from different lymphatic tissue by flow cytometry. Data shown are numbers of $\alpha_5\beta_1$ integrin-positive memory B cells from (C, D) tonsils, (F, G) peripheral blood and (I, J) lymph nodes. (E, H, K)

Relative fluorescence intensity of β_1 integrin on memory B cells from different lymphatic tissue. Data shown are mean ± SD from 3-4 biological replicates. *P* values were calculated by using the Mann-Whitney t test.

Figure 4. Integrin $\alpha_5\beta_1$ is required for efficient attachment and entry of EBV on memory B cells

(A) Infection susceptibility of $\alpha_5\beta_1$ integrin-positive and negative tonsillar memory B cells. Cells were either stained for the expression of $\alpha_5\beta_1$ integrin 24 hours post EBV inoculation or were sorted by fluorescence-activated cell sorting before infection with EBV. (B) Inhibition of cellular receptors required for EBV infection (CD21 and HLA class II) and $\alpha_5\beta_1$ integrin on memory B cells from tonsils using different concentrations of blocking antibodies and combinations. (C) Inhibition of the viral glycoproteins gp350/220 and gp42 in combination with competition inhibition of recombinant soluble β_1 integrin using tonsillar memory B cells. Data shown are from one representative experiments of two replicates.

Figure 5. EBV binding to integrins via BMRF2 initiates signaling cascades mandatory for virus entry into memory B cells.

(A, B) Phosphorylation of FAK, c-Src, p85α, and cofilin following binding of (A) B95.8wt or (B) BMRF2ko EBV to isolated tonsillar memory B cells. (C) Initiation of actin filament reorganization triggered by binding of EBV, Fibronectin, heat-inactivated EBV or BMRF2ko EBV to isolated tonsillar memory B cells.

Figure 6. Activation of the integrin signal transduction pathway is crucial for EBV entry into memory B cells

(A, B) Inhibition of signaling molecules *c*-Src, PI_3 kinase, FAK, activated downstream of integrin impact the susceptibility of tonsillar memory B cells to EBV infection. Cytochalasin D was included to show the dependence of infection susceptibility of memory B cells on actin cytoskeletton depolymerization.

Figure 7. Proposed model for the attachment and entry of EBV into memory B cells. EBV binding to CD21 on the cell surface triggers receptor-mediated endocytosis and, at the same time initiates integrin signal transduction pathways. While EBV triggers membrane fusion with the endosomal membrane via interaction of the viral glycoprotein complex gH/gL/gp42 and host cellular HLA-DR,DP,DQ, the activated integrin signaling cascade leads to subsequent activation of actin filament rearrangement and microtubule formation thereby enhancing transport of the viral capsid to the nucleus.

Figure 1

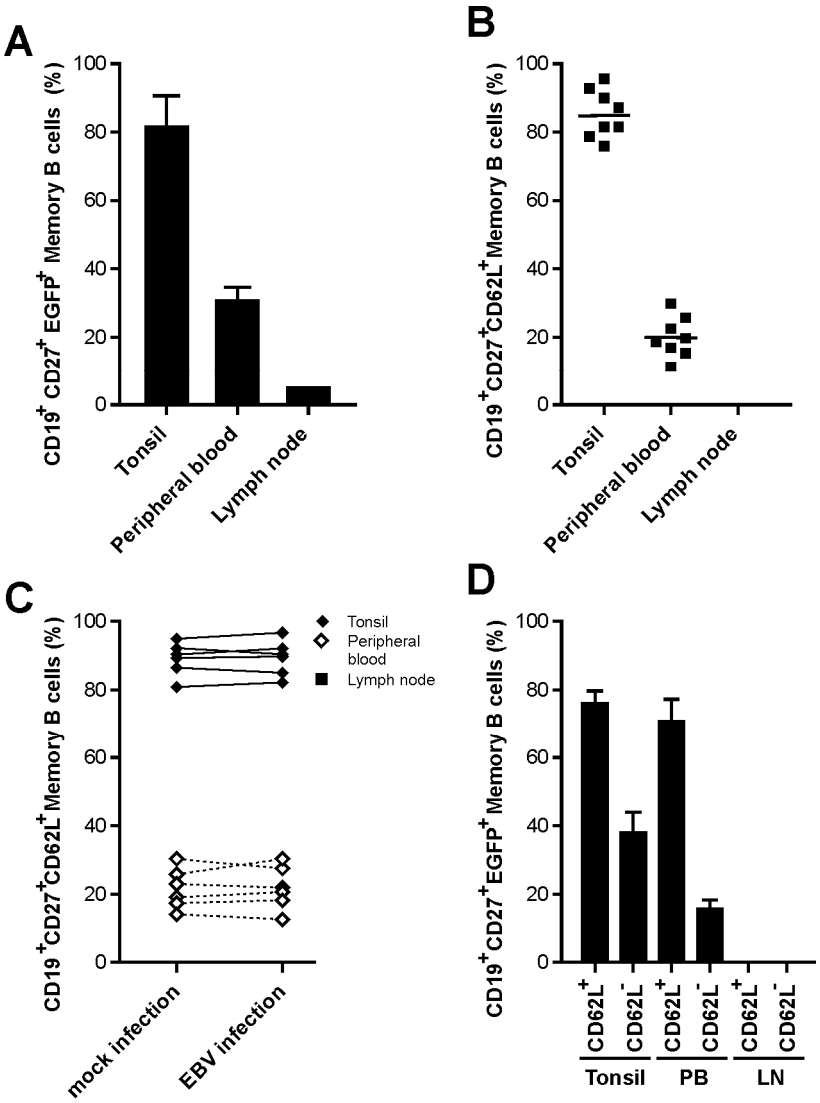

A
CD19+ CD27+ EGFP+ Memory B cells (%)
100
80
60
40
20
0
Tonsil
Peripheral blood
Lymph node
B
CD19+CD27+CD62L+Memory B cells (%)
100
80
60
40
20
0
Tonsil
Peripheral blood
Lymph node
C
CD19+CD27+CD62L+ Memory B cells (%)
100
80
60
40
20
0
Tonsil
Peripheral blood
Lymph node
mock infection
EBV infection
D
CD19+CD27+EGFP+ Memory B cells (%)
100
80
60
40
20
0
CD62L+
CD62L-
CD62L+
CD62L-
CD62L+
CD62L-
Tonsil
PB
LN

Figure 2

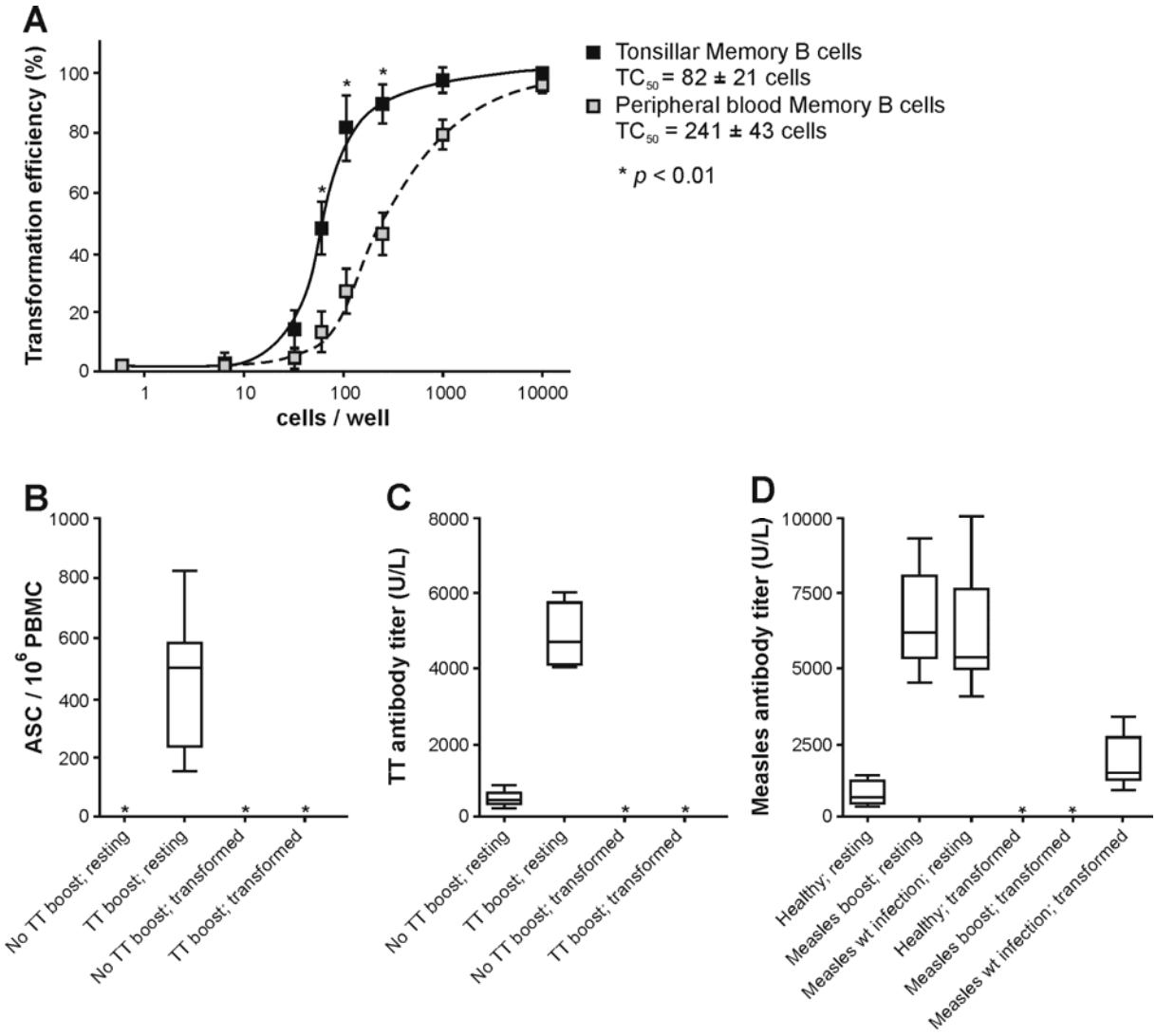

A
Transformation efficiency (%)
cells / well
Tonsillar Memory B cells
TC50 = 82 ± 21 cells
Peripheral blood Memory B cells
TC50 = 241 ± 43 cells
* p < 0.01
B
ASC / 10^6 PBMC
No TT boost; resting
TT boost; resting
No TT boost; transformed
TT boost; transformed
C
TT antibody titer (U/L)
No TT boost; resting
TT boost; resting
No TT boost; transformed
TT boost; transformed
D
Measles antibody titer (U/L)
Healthy; resting
Measles boost; resting
Measles wt infection; resting
Healthy; transformed
Measles boost; transformed
Measles wt infection; transformed

Figure 3

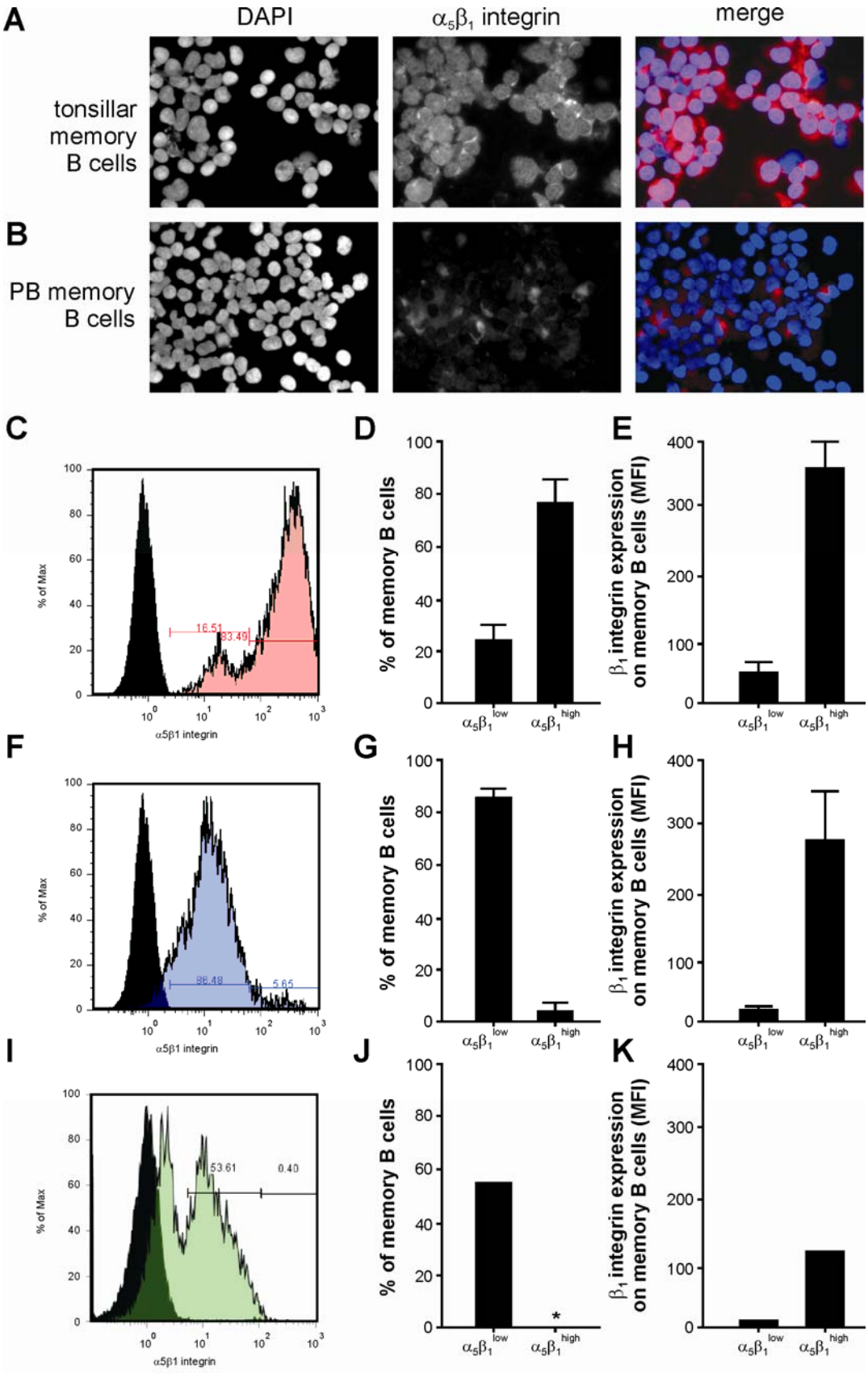

A
DAPI
$\alpha_5\beta_1$ integrin
merge
tonsillar memory B cells
B
PB memory B cells
C
D
E
F
G
H
I
J
K
% of Max
α5β1 integrin
16.51
93.49
86.48
5.65
53.61
0.40
% of memory B cells
β_1 integrin expression on memory B cells (MFI)
$\alpha_5\beta_1^{low}$
$\alpha_5\beta_1^{high}$
*

Figure 4

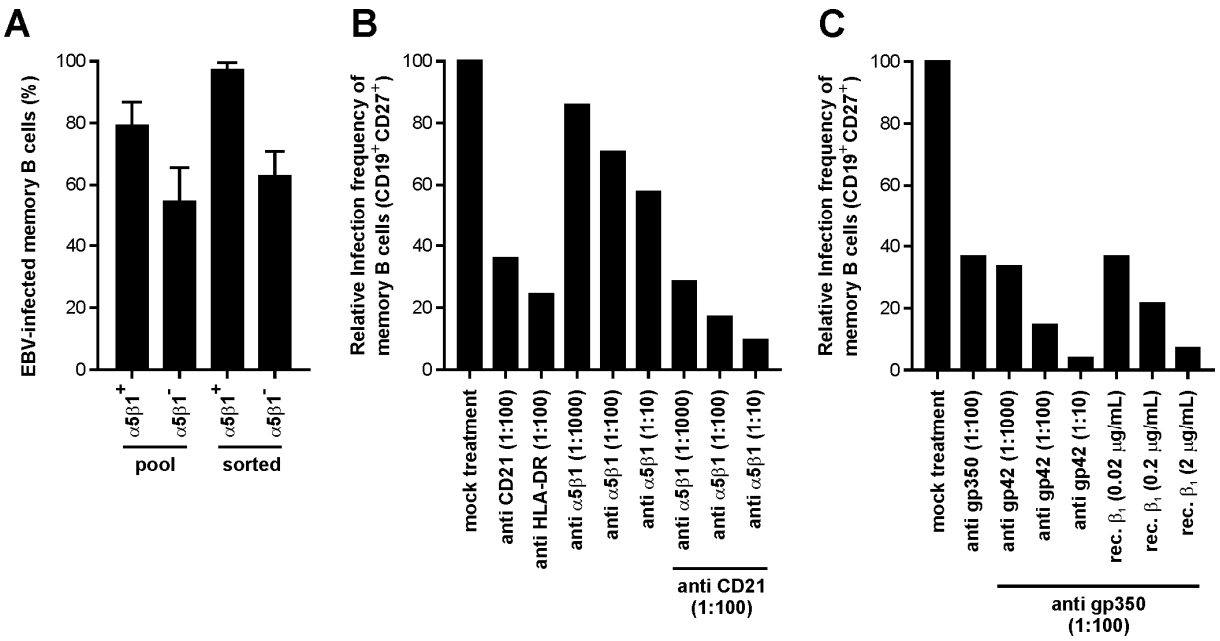

A
EBV-infected memory B cells (%)
100
80
60
40
20
0
α5β1+
α5β1-
α5β1+
α5β1-
pool
sorted
B
Relative Infection frequency of memory B cells (CD19+CD27+)
100
80
60
40
20
0
mock treatment
anti CD21 (1:100)
anti HLA-DR (1:100)
anti α5β1 (1:1000)
anti α5β1 (1:100)
anti α5β1 (1:10)
anti α5β1 (1:1000)
anti α5β1 (1:100)
anti α5β1 (1:10)
anti CD21 (1:100)
C
Relative Infection frequency of memory B cells (CD19+CD27+)
100
80
60
40
20
0
mock treatment
anti gp350 (1:100)
anti gp42 (1:1000)
anti gp42 (1:100)
anti gp42 (1:10)
rec. β1 (0.02 μg/mL)
rec. β1 (0.2 μg/mL)
rec. β1 (2 μg/mL)
anti gp350 (1:100)

Figure 5

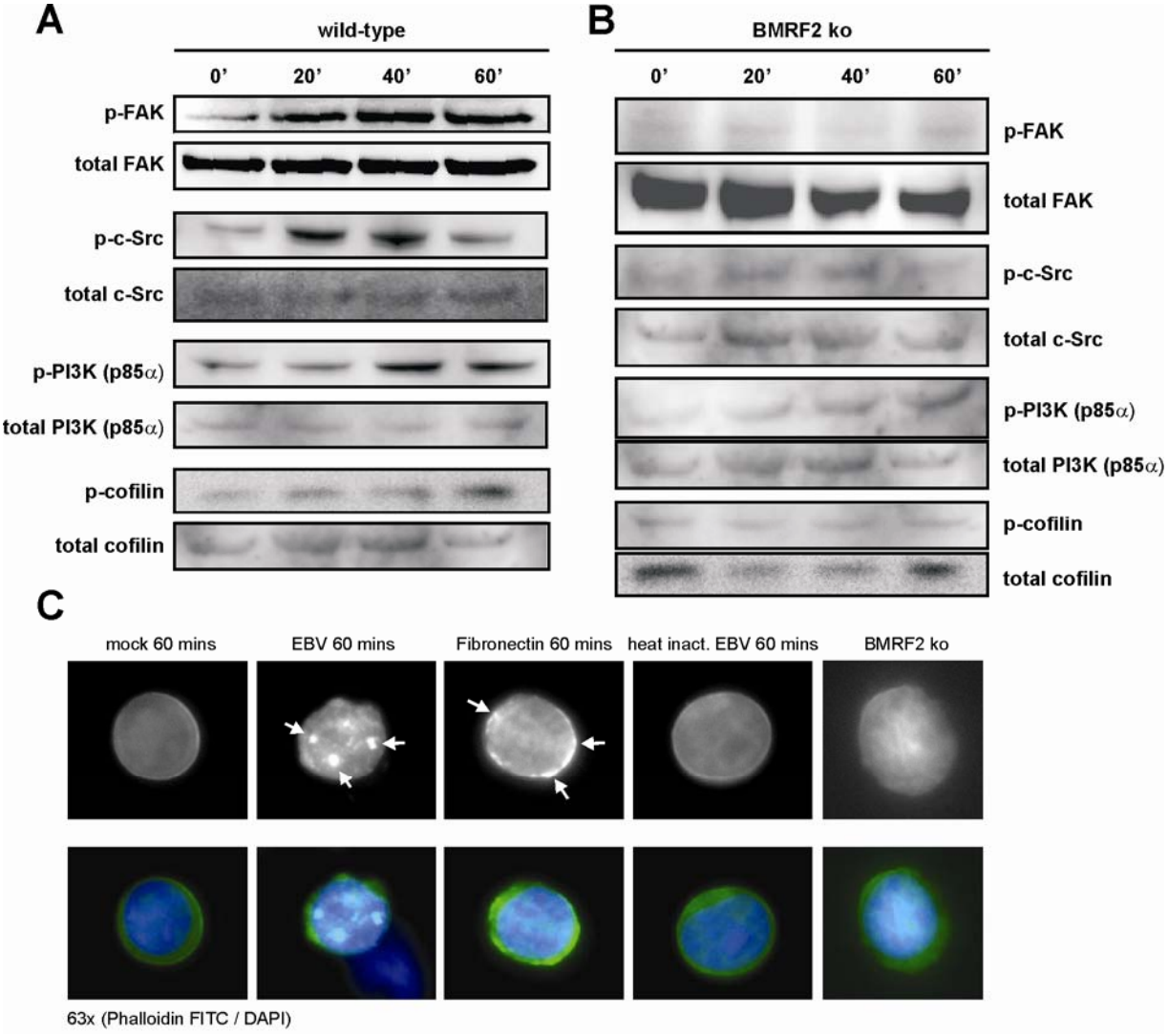

A
wild-type
0'
20'
40'
60'
p-FAK
total FAK
p-c-Src
total c-Src
p-PI3K (p85α)
total PI3K (p85α)
p-cofilin
total cofilin
B
BMRF2 ko
0'
20'
40'
60'
p-FAK
total FAK
p-c-Src
total c-Src
p-PI3K (p85α)
total PI3K (p85α)
p-cofilin
total cofilin
C
mock 60 mins
EBV 60 mins
Fibronectin 60 mins
heat inact. EBV 60 mins
BMRF2 ko
63x (Phalloidin FITC / DAPI)

Figure 6

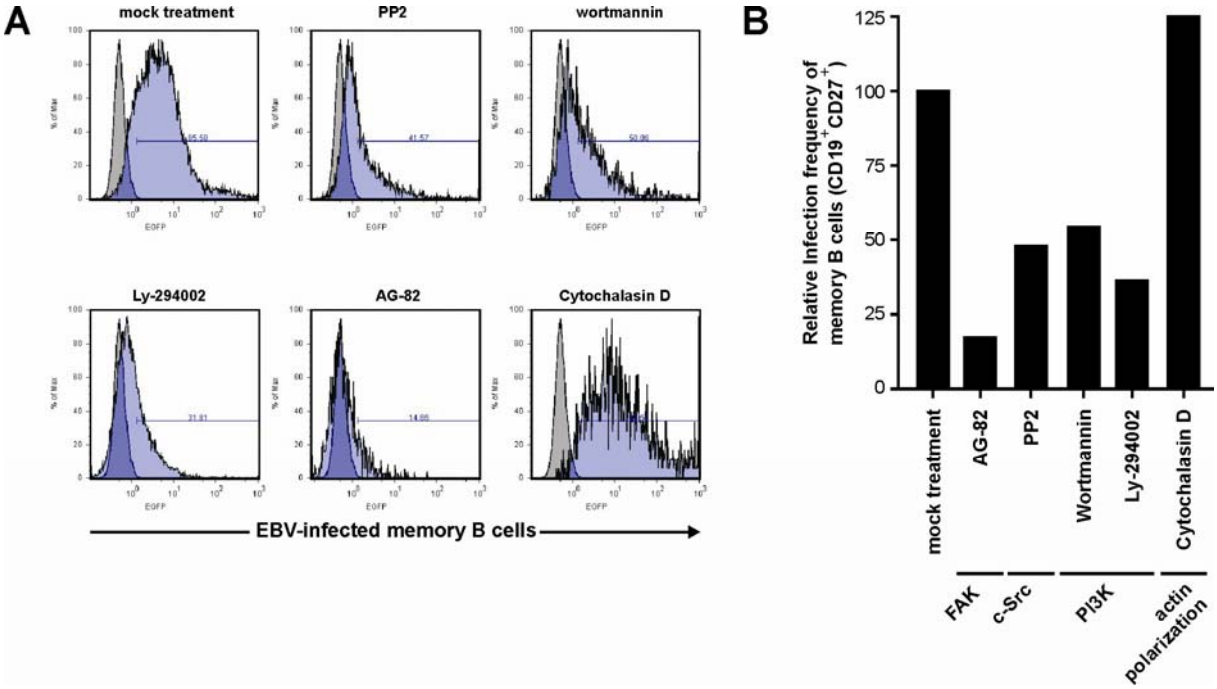
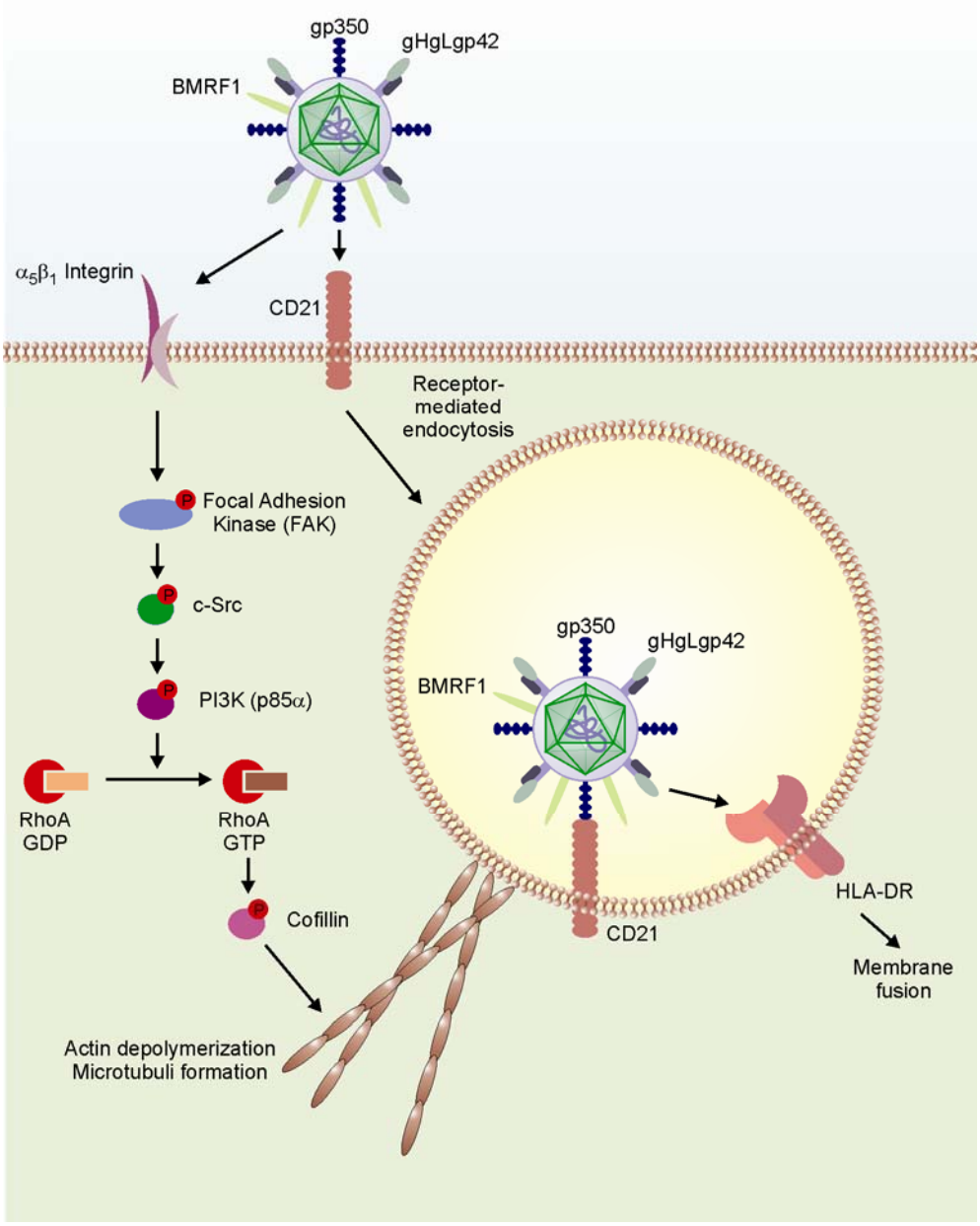

A
mock treatment
PP2
wortmannin
Ly-294002
AG-82
Cytochalasin D
EBV-infected memory B cells
B
Relative Infection frequency of memory B cells (CD19+CD27+)
125
100
75
50
25
0
mock treatment
AG-82
PP2
Wortmannin
Ly-294002
Cytochalasin D
FAK
c-Src
PI3K
actin polarization

Figure 7


gp350
gHgLgp42
BMRF1
$\alpha_5\beta_1$ Integrin
CD21
Receptor-mediated endocytosis
Focal Adhesion Kinase (FAK)
c-Src
PI3K (p85α)
RhoA GDP
RhoA GTP
Cofillin
Actin depolymerization
Microtubuli formation
gp350
gHgLgp42
BMRF1
CD21
HLA-DR
Membrane fusion

Epstein-Barr Virus Triggers Integrin and Focal Adhesion Kinase-Mediated Signalling During Binding to and Infection of Epithelial Cells

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Running title: EBV induced signalling in epithelial cells

Word count:

Abstract

The γ-herpesvirus Epstein-Barr (EBV) preferentially infects human B and epithelial cells and is associated with a pathogenic role in neoplasia of both types. While EBV infection of B cells is well-characterized, mechanisms of entry into epithelial cells are poorly understood. Binding of the EBV glycoprotein BMRF-2 to integrins of the β1 family plays a crucial role in the epithelial cell infection process. Nevertheless, the mechanisms by which integrins facilitate EBV entry are not defined. We investigated the events triggered by EBV binding to epithelial cells in a tissue culture system using the model cell line AGS. We demonstrate that binding of EBV to integrin and subsequent intracellular signalling are crucial for augmenting EBV infection and that EBV triggers signalling events leading to activation of focal adhesion kinase (FAK). FAK and its downstream effectors PI3K and c-Src were essential for enhancing EBV infection as well as for EBV-mediated changes in the cytoskeleton. EBV binding induced activation of the regulatory small GTPase RhoA leading to a disassembly of cortical actin and the formation of actin stress fibres; furthermore, EBV caused a reorganization and stabilization of microtubuli. These data suggest pathways that may facilitate EBV entry into epithelial cells and further define the EBV-host interaction. A deeper understanding of such mechanisms is key for understanding EBV biology and notably pathogenesis of EBV-associated diseases.

Introduction

The γ-herpes virus Epstein-Barr (EBV) is a dual cell-tropic virus that infects B cells and nasopharyngeal and gastric epithelial cells where it can establish latency (reviewed in Faulkner et al. 2000). Latent EBV has been attributed a pathogenic role in several types of neoplasia including B cell lymphomas in immunocompromised patients (reviewed in Young and Rickinson 2004). salivary gland carcinomas, gastric adenocarcinomas, and nasopharyngeal carcinoma (NPC) (Raab-Traub 2002). In all of these tumors, each cell is infected with EBV.

In vitro models for EBV infection have led to a detailed characterization of the virus entry process in B cells (reviewed in Hutt-Fletcher 2007). Here, infection is initiated by attachment of EBV through binding of the viral envelope glycoprotein (env gp) gp350/220 to its cell surface receptor CD21 (also known as complement receptor type 2 (CR2)) (Nemerow et al. 1989) and subsequent endocytosis of the ligand-receptor complex (Tanner et al. 1987). The initial interaction tethers EBV and promotes the subsequent binding of a second glycoprotein, gp42, which is found in a trimeric complex with the glycoproteins gH and gL, to HLA class II (Fingeroth et al. 1984; Tanner et al. 1987). gp42 binding to HLA class II induces a conformational change in the trimeric protein complex, which triggers the fusion machinery into an active state (Li et al. 1995). Fusion of EBV with B cells is mediated by the gH/gL complex and an additional protein, gB (McShane and Longnecker 2004).

EBV infection of epithelial cells is quite different from infection of B cells and remains mostly enigmatic. Epithelial cells do neither express CD21 nor HLA class II. Hence, binding of env gp350 and gp42 is not possible. While a trimeric complex of gH/gL and gp42 is needed for infection of B cells, addition of soluble gp42 to a dimeric gH/gL complex has an inhibitory effect on EBV's fusion with epithelial cells (Wang et al. 1998). To enable dual cell tropism, the virus thus carries both dimeric and trimeric complexes, the ratio of which

depends on the cellular origin of the virus (Borza and Hutt-Fletcher 2002). The minimal viral requirement for EBV fusion with epithelial cells is the expression of the glycoproteins gH, gL and gB. Though, close cell–cell contact and the expression of the glycoproteins are not sufficient, hinting at the need for a receptor or attachment factor on the target cell itself (McShane and Longnecker 2004). The finding that binding to epithelial cells can be blocked by antibodies to gH/gL suggests a critical role of this glycoprotein complex in receptor binding. Taking into account the inhibitory effect of soluble gp42, the cell domain which interacts with gp42 in the trimeric complex most likely exhibits important binding receptor function (Kirschner et al. 2006). Thus, while the core fusion function of gH/gL remains identical for B cells and epithelial cells, the way in which it is triggered into a functionally active state by binding receptors differs considerably (Wu et al. 2005).

In addition to the putative gH/gL receptor, other cellular factors might be involved in EBV binding to epithelial cells. A recently identified EBV env glycoprotein, BMRF-2, has the ability to bind to integrins of the β_1 family through its RGD (Arg-Gly-Asp) motif (Xiao et al. 2008). An inhibition of this interaction substantially decreases infection, implicating a crucial role for integrins in EBV binding (Tugizov et al. 2003). BMRF-2 is expressed at the basolateral membranes of EBV-infected epithelial cells and may thus also play a role in the direct cell to cell spread of EBV in epithelia (Xiao et al. 2007). Nevertheless, the mechanisms by which the BMRF-2-integrin interaction facilitates EBV entry and infection are not defined.

Integrins are a family of heterodimeric receptors composed of non-covalently linked class I transmembrane α and β subunits (Giancotti 1999). In man at least 24 different receptor combinations are found that can be grouped into three sub-classes according to their ligand-specificity, one of which is defined through its RGD-binding properties, and interacts with extracellular matrix (ECM) components like Fibronectin and Vitronectin (reviewed in Wiesner 2005). The most basic property of integrins is to provide a mechanically strong

connection between cells and the ECM. This connection mediates a wide array of functions such as activation of endocytosis, attachment, motility, regulation of gene expression, cell survival, cell cycle progression, apoptosis, differentiation and regulation of the cytoskeleton (Giancotti 1999). When active integrins bind to their ECM ligands, their cytoplasmic tails recruit proteins that trigger the assembly of micrometer-sized structures termed cell matrix adhesion complexes (MACs), which can be further differentiated into focal adhesions (FA). FAs are built up by integrins and numerous signalling molecules, such as focal adhesion kinase (FAK), Src kinases, phosphoinositol-3-kinases (PI3Ks) and cytoskeletal proteins like talin, paxillin, vinculin and α-actinin. FAs thus link the ECM proteins on the outside of the cell with the cytoplasmic actin cytoskeleton on the inside (Clark and Brugge 1995; Parsons 1996; Giancotti 1999; Wiesner 2006). The signalling cascades within FA eventually lead to the recruitment and activation of Rho GTPases and their effectors, which in turn regulate the activity of actin nucleation factors (Ridley 2001), such as the Arp2/3 complex and the forming protein family (Pollard 2003; Higgs 2005).

It has been shown for several viruses that binding to the plasma membrane and in particular to integrins triggers intracellular signalling events preparing the cell for invasion. These signalling events may activate endocytosis leading to the uptake of the virus as in the case of adenovirus 2 (Meier et al. 2002), or simply facilitate fusion with the plasma membrane (Cheshenko et al. 2003). In the case of herpes viruses, several examples of integrin-mediated signalling exist. Human cytomegalovirus (HCMV) uses $\alpha_v\beta_3$ integrins as co-receptors and triggers signalling leading to RhoA down regulation, actin disassembly and facilitated capsid transport to the nucleus (Wang et al. 2005). Karposi's sarcoma-associated herpesvirus (KSHV) induces an integrin-dependent focal adhesion kinase-Src signalling pathway, leading to activation of Rho GTPases and changes in the cytoskeleton, which

include increased formation of microtubules serving also in this case for capsid transport to the nucleus (Sharma-Walia et al. 2004; Naranatt et al. 2005; Raghu et al. 2007).

This study was undertaken to investigate whether EBV binding to integrins also triggers signalling processes, which facilitate infection with the virus in any way. We used virus binding to and infection of the epithelial gastric adeno-associated carcinoma cell line (AGS) as a model to study EBV infection of epithelial cells and could show several lines of evidence that not only binding to but also integrin signalling is essential. We further demonstrated that EBV interaction with integrins leads to activation of focal adhesion kinase and down-stream effectors, which culminates in RhoA activation and changes of the cytoskeleton that may facilitate virus entry.

Materials and Methods

Cells and culture. The EBV-infected marmoset cell line B95.8 EBfaV-GFP was maintained in RPMI 1640 medium (Gibco, Basel, Switzerland) supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin, referred to hereafter as complete RPMI. The human adeno-associated gastric carcinoma cell line AGS (ATCC CRL-1739) was maintained in Ham's F-12 medium (Gibco) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1% L-glutamine, referred to hereafter as complete Ham's F-12, and split twice a week.

Preparation of EBV stock. B95.8 EBfaV-GFP cells were seeded at a density of 10^6 cells/ml and were stimulated to release virus by culture for 4 days in complete RPMI containing 50 ng/ml of 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma-Aldrich, Buchs, Switzerland). Cell suspensions were centrifuged at 400 x g for 5 min. Supernatants were passed through a 45-µm-pore-size cellulose acetate filter (Millipore, Zug, Switzerland) and stored at -80°C. For purification and concentration the supernatants were centrifuged for 4h at 4°C and 17000 rpm to pellet the virus. The supernatants were poured of and virus pellets resuspended in TBSal (200 mM NaCl, 2.6 mM KCl, 10 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 1.8 mM $CaCl_2$)) and frozen in aliquots at -80°C. The virus was titrated on primary B cells isolated from tonsilar mononuclear cells (see ref Dorner for details) to determine infectivity.

Plasmids. The plasmids pCMVIL2R encoding the full-length tac subunit (CD25) of the IL2-receptor and pCMVIL2Rβ_1 encoding the extracellular and transmembrane domains of the tac subunit of IL-2 receptor connected to the wild-type β_1 integrin cytoplasmic domain acting as dominant negative have been described before (LaFlamme 1994 JCB) and were obtained from Susan LaFlamme (Albany Medical College, Albany, NY, USA).

Antibodies and reagents. Monoclonal antibodies directed against focal adhesion kinase and phosphorylated focal adhesion kinase (Y397) were purchased from BD

Biosciences (Basel, Switzerland). Monoclonal antibodies against tubulin (B-5-1-2) and acetylated tubulin (6-B11-1) were from Sigma-Aldrich. Polyclonal rabbit antibody against β-actin was from BioConcept (Allschwil, Switzerland). CD25 MicroBeads and mouse anti-CD25-PE antibodies for MACS sorting and subsequent FACS analysis were purchased from Miltenyi Biotech (Bergisch-Gladbach, Germany). The monoclonal antibody directed against RhoA was part of the Rho Activation Assay Biochem Kit from Cytoskeleton (Denver, CO, USA). Alexa-Fluor coupled secondary anti-mouse and anti-rabbit reagents were from Invitrogen (Basel, Switzerland) and HRP-coupled secondary anti-mouse and anti-rabbit reagents from Pierce Biotechnology (Rockford, USA). The soluble recombinant β_1 integrins (CD29) were synthesized at RayBiotech (Norcross, USA). FITC-coupled Phalloidin, Fibronectin, the PI3K inhibitors Ly294002 and Wortmannin as well as the actin depolymerising agent Cytochalasin D were all purchased from Sigma-Aldrich. The tyrosine-kinase inhibitor AG82 (Tyrphostin A25) and the c-Src inhibitor PP2 were both from Calbiochem (ordered at Merck, Nottingham, UK).

Transfection and cell sorting. AGS cells were transfected using the Nucleofector 2 from Amaxa Biosystems (Cologne, Germany) according to the manufacturer's protocol. In brief, the cells were trypsinized and washed in PBS and pelleted at 1,500 rpm/5min. 10^6 cells were resuspended in 100 μl buffer V (Amaxa) and mixed with 2 μg plasmid DNA. The cells were pulsed with programme B-023, taken up in warm complete Ham's F-12 and plated for experiments or subsequent sorting. Transfected cells were processed for positive MACS sorting with CD25 Microbeads II from Miltenyi according to the manufacturer's protocol after 24 h. The purity of sorted populations was checked by flow-cytometry using compatible anti-CD25-PE from the same company. Typically, sorting of a population with 30% expressing cells yielded a purity of 70-80%.

EBV infection and binding. To determine infection efficiencies after treatment of cells with inhibitors at 37°C for 30 min or 1h as specified, high-speed centrifugation purified virus from TPA induced B95.8EBfaV-GFP cells was used to infect AGS cells by spinoculation. In detail, 50 µl of concentrated virus was added to 3 x 10^5 AGS cells in 6-well plates with 500 µl complete Ham's F-12 and the cells were centrifuged for 1 h at 800 x g at room temperature in presence of the inhibitor where required. After spinoculation, cells were washed in phosphate-buffered saline (PBS) and supplied with fresh complete medium. Two days later, the infection of cells was assessed by flow cytometry quantifying the expression of GFP. To assess the activation of signalling pathways and changes in the cytoskeleton by Western blot and immunofluorescence methods AGS cells were serum-starved for 24h at 37°C. 50 µl purified virus was added to the cells in 500 µl serum-free Ham's F-12 and incubated at 37°C for 5 to 60 min as indicated. Cells were put on ice, washed in phosphate buffered saline and processed for subsequent assays.

Flow cytometry. Flow cytometry was carried out on a Cytomics FC500 instrument (Beckman Coulter, Nyon, Switzerland) with FlowJo software, used in accordance with the instructions of the manufacturer (Treestar, Ashland, USA).

Immunofluorescence. AGS cells grown on glass coverslips in 24-well plates were fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min. the fixation was stopped by two washes in Hepes-buffered serum-free Ham's F-12 and subsequent incubation at 4°C in phosphate-buffered saline (PBS) containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$. Cells were permeabilized with a buffer containing 0.05% Saponin, 10% fetal bovine serum, 15 mM Glycin, and 10 mM Hepes in PBS for 15 min and subsequently stained in the same buffer with added antibodies or Phalloidin. Each antibody incubation step was followed by five washes in permeabilization buffer, the last one additionally by two washes in PBS. The coverslips were briefly rinsed in deionised water and mounted onto glass slides in ProLong

Gold Antifade reagent containing DAPI (Invitrogen). Samples were analyzed by epifluorescence with a Zeiss Axioskope 2 and AxioVision 4.4 software (Carl Zeiss Vision GmbH, Feldbach, Switzerland). Images were assembled with Adobe Photoshop 6.0.

Western blotting. Cells were lysed on ice in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulphate, 0.5% sodium deoxy cholate) supplemented with complete mini protease inhibitor cocktail (Roche Applied Sciences, Rotkreuz, Switzerland), sodium fluoride and 1 mM sodium orthovanadate (Sigma-Aldrich) for 15 min. Cell debris and nuclei were removed by centrifugation at 12,000 rpm/10 min/4°C and proteins resolved by 10% SDS PAGE before transfer to nitrocellulose membranes. Antibody incubations were generally done in PBS with 0.1% Tween and 5% fat free dried milk. For the detection of primary antibodies horseradish peroxidase-labelled goat anti-mouse or anti-rabbit reagents from Pierce were used. Signal detection was performed by using the enhanced-chemiluminescence substrate from Pierce and scanning directly with the ChemiGenius imaging system (SynGene, Cambridge, UK). Signals were quantified with the GeneTools 3.1 image analysis software (SynGene).

RhoA pull-down assay. Quantification of RhoA-GTP was performed using the Rho Activation Assay Biochem Kit from Cytoskeleton (Denver, USA) according to the manufacturer's instructions. In brief, cells treated with EBV were lysed on ice in the provided buffer and debris removed by centrifugation. All samples were snap-frozen in liquid nitrogen straight after lysis to prevent GTP hydrolyis. Protein concentrations were equalized and 800 μg of total lysate used for pull-down with 15 μg of rhotekin-sepharose beads. Eluates from the pull-down were subsequently analyzed by SDS-PAGE and Western blotting for RhoA in comparison to total lysate. For positive and negative pull-down controls, lysates were loaded with GDP or the non-hydrolysable GTPγS prior to addition of beads.

Results

EBV infection of epithelial cells is augmented by binding to integrin and integrin signalling.

EBV can enter tongue and pharyngeal epithelial cells through three different CD21/independent pathways, either by cell-to-cell spread directly across lateral membranes, by transfer from infected B cells to apical membranes or as cell-free virion by direct infection through basolateral membranes (Tugizov et al. 2003). Infection with cell-free virions is, at least in part, mediated by a direct interaction of the viral glycoprotein BMRF-2 with integrins of the β_1-family, which facilitates binding of the virus to the cell (Tugizov et al. 2003; Xiao et al. 2008). Nevertheless, it has not been demonstrated, whether integrins only serve a binding role or whether EBV is triggering integrin-mediated signalling facilitating it's own entry. To show that EBV binding to integrins is indeed crucial for augmenting infection, we pre-treated purified B95.8 EBfaV-GFP virions with increasing concentrations of soluble recombinant β_1 integrins to block the binding sites on BMRF-2. The pre-treated EBV was subsequently used to infect the epithelial model cell line AGS by spinoculation. The number of infected cells was assessed by flow cytometry detecting the amount of GFP expression after 48h. EBV treatment with soluble integrin significantly reduced infection in a concentration-dependent manner (Fig. 1A) indicating a need for integrin binding on the host cell. We thus wanted to investigate the implications of integrin signalling on infection efficiency using dominant negative constructs: fusion proteins joining the β_1 integrin cytoplasmic domain to the transmembrane and extracellular parts of the interleukin 2 receptor α-subunit (IL2R) have been described previously (LaFlamme 1994; Bodeau AL 2001), and are inhibiting endogenous integrin signalling by binding to down-stream effector proteins. We used plasmids encoding the dominant negative (DN IL2Rβ1) or the full-length interleukin 2 receptor α-subunit (IL2R, as a control) to nucleofect AGS cells and obtained approximately

30% of expressing cells as determined by flow-cytometry and IL2R staining (data not shown). To enrich the expressing population we sorted the nucleofected cells using MACS-beads coupled to antibodies recognizing the extracelullar part of the IL2R, what yielded 70-80% purity. The selected cells were subsequently spinoculated with EBV (B95.8 EBfaV-GFP) and the infection analyzed by flow cytometry of GFP expression compared to untransfected cells (Fig. 1B). While infection remained unchanged in cells expressing the IL2R control, it was reduced to below 50% of controls in cells expressing the dominant negative construct, indicating that integrin signalling essentially contributes to augment infection.

EBV triggers the activation of focal adhesion kinase.

The non–receptor protein tyrosine kinase focal adhesion kinase (FAK) plays a central role in signalling through integrins and a variety of other receptors (reviewed in Parsons 2003). It links signals from the extracellular matrix (ECM) to PI3kinases, Src kinases and regulatory elements of the cytoskeleton, and its activation leads to autophosphorylation of Y397 (reviewed in Wiesner 2005). To test whether EBV binding to integrins on AGS cells leads to activation of FAK, we incubated cells for varying times with EBV or the ECM integrin ligand Fibronectin as a positive control at 37°C, and analyzed them by immunofluorescence staining with an antibody specific for FAK phosphorylated at Y397. Distinct spots of staining were observed after 1h Fibronectin treatment (Fig. 2A second panel from left), which were not visible in control cells. Treatment of cells with EBV for 15 min led to a weak signal for phosphorylated FAK at the plasma membrane that grew substantially stronger after incubation with EBV for 60 min and was distributed evenly along the plasma membrane of the cells, indicative of accumulative activation. To quantify FAK activation, cells treated with either Fibronectin or EBV were lysed and processed for Western blotting with the phophorylated Y397 FAK specific antibody. Staining of the same blots for β-actin was used as a loading control. Quantification of a representative experiment out of several

(Fig. 2B), showed an approximately four-fold activation of FAK after Fibronectin treatment. In agreement with the immunofluorescence experiment, a weak activation (1.3 fold) was observed after 15min EBV treatment, which grew stronger (2 fold) after 60 min. We concluded that EBV binding to AGS cells triggers activation of FAK.

To address a role for FAK in EBV epithelial cell infection, the protein tyrosine kinase inhibitor AG82 was used, which has been shown to inhibit FAK activity in some cell types (Tsuda et al. 1997). AGS cells were incubated for 30 min with increasing concentrations of AG82 and subsequently spinoculated with EBV in presence of the inhibitor. Infection efficiency was determined by flow-cytometry after 2 days. While 5μM of AG82 did not affect EBV infection, a reduction to around 60% of controls was observed at 10 μM and 25 μM, respectively (Fig. 2C). Higher concentrations showed a weaker effect, probably due to non-specific inhibition of phospho-tyrosine kinases with contrasting functions, and concentrations above 50 μM were toxic for the cells (not shown).

EBV infection is dependent of signalling pathways downstream of FAK.

Phosphorylation of FAK creates a binding site for Src and enables interactions with the SH2 domain of phosphoinositide 3-kinase (PI3K), activating down-stream signalling pathways (reviewed in Wiesner 2005). We thus wanted to investigate whether these pathways are essential for augmenting EBV infection. The function of PI3K is irreversibly inhibited by Wortmannin, which interferes with its catalytic subunit, and reversibly by Ly294002 (Araki et al. 1996), while function of c-Src family kinases can be blocked with 4-Amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-d]pyrimidine (PP2). We pre-treated AGS cells with above three inhibitors for 1 h and subjected them to spinoculation with EBV in presence of the inhibitors. The cells were then incubated at 37°C until infection became visible by GFP expression after 2 days. We observed a clear infection decrease after treatment with Ly294002 (less than 5% of control) or Wortmannin (40% of control) (Fig. 3) indicating that

signalling processes requiring PI3K are essential. Blocking the key modulator kinases of the c-Src family with PP2 also led to a reduction of infection to approximately 40% of control (Fig. 3), suggesting a need for c-Src signalling for augmenting infection.

EBV triggers a reorganization of the actin cytoskeleton facilitating infection.

Among the hallmarks of integrin interaction with ligands are reorganization and remodelling of the actin cytoskeleton, which are controlled by small GTPases of the Rho family such as RhoA, Rac and Cdc42. RhoA mediates the formation of stress fibers, elongated actin bundles traversing the cell and promoting cell attachment to the ECM (Tapon 1997), while Rac and Cdc42 are responsible for the formation of lamellipodia or ruffles and filopodia, respectively. These GTPases can be activated upon FAK and Src induction via PI3Ks and have effects on FA assembly through actin filament reorganization. Activation of FAK by EBV binding, as well as the above suggested involvement of PI3K and c-Src signalling in EBV infection, prompted us to investigate, whether the EBV induced signalling cascades could also mediate cytoskeletal reorganization.

AGS cells were serum-starved for 24h and subsequently treated with serum-free Ham's F12 (controls) or with serum-free Ham's supplemented with 40μg/ml Fibronectin or EBV for 15 min or 60 min at 37°C. The cells were subsequently fixed, permeabilized and stained with FITC-coupled Phalloidin for analysis by epifluorescence. Serum-starved control cells exhibited strong peripheral F-actin staining along the cell edges, indicative of cortical actin fibres. The margins of the cells were well defined and smooth (Fig. 4A top left panel). Treatment of cells with Fibronectin induced the polymerization of actin, visible as stress fibres inside the cell, while part of the strong cortical actin was still present (Fig. 4A, top right panel). 15 min of incubation with EBV led to a much more pronounced stress fibre pattern, stretching throughout the whole cells. At this point the cortical actin had completely

disappeared and the edges of the cells appeared less even (Fig.4A bottom left panel), indicative of substantial actin reorganization. After 60 min of incubation with the virus the stress fibres were still visible, but part of the cortical actin staining had reappeared, what suggested a decrease of the EBV-induced signal and return to the original state of the cells (Fig.4A, bottom right panel). We thus concluded that EBV binding induced changes in the actin cytoskeleton, which resulted in a shift from a predominant cortical form to intracellular stress fibres.

We next wanted to investigate whether the demonstrated changes in the actin cytoskeleton are indeed mediated by in above experiments suggested FAK, PI3K and c-Src involving pathways. To this end we incubated serum starved AGS cells with non-toxic concentrations of the corresponding inhibitors of the kinases, AG82, Ly294002 and PP2 respectively, before triggering actin reorganization with EBV. Actin staining in control cells showed that the inhibitors did not markedly change the morphology of the cells (Fig. 4B top row). While some cells showed a slightly more rounded shape and retracted from the coverslips, most remained attached and exhibited a strong cortical actin pattern reminiscent of the untreated control cells in Fig 4A. In contrast, EBV incubation did not lead to pronounced stress fibre formation as observed in cells without inhibitors earlier. After 15 min the cortical actin was still partly present, particularly in AG82 or PP2 treated cells, and fibres had only formed around the edges of the cells to a very small extent. This state persisted even after 60 min of EBV treatment (Fig. 4B lower panels). We thus concluded that AG82, Ly294002, or PP2 efficiently inhibited EBV-mediated stress fibre formation and that thus the trigger may be transmitted by pathways including FAK, PI3K, and c-Src.

We subsequently wanted to analyze, whether the disruption of actin fibres with non-toxic doses of Cytochalasin D affected EBV infection. AGS cells were thus treated with Cytochalasin D for 1h prior to EBV infection by spinoculation in the presence of the

disrupting agent. We surprisingly observed that infection efficiency was increased by almost 4-fold after treatment compared to mock (solvent only) treated cells (Fig. 4C). This result led to the speculation that not the formation of stress fibres, but rather the disassembly of the cortical actin was required for the infection process.

EBV triggers activation of RhoA

Considering the substantial changes in the actin cytoskeleton by EBV binding to AGS cells, we wanted to investigate, whether EBV had a direct influence on the activation status of the actin stress fibre regulating small GTPase RhoA. AGS cells serum starved for 24h were thus treated with either medium control or EBV for times ranging from 5 min to 60 min and subsequently washed and directly lysed. The amount of total protein in the samples was equalized and a total lysate probe of each sample analyzed by Western blotting for the expression of total RhoA (Fig. 5A, lower panel). Equal amounts of lysates from each sample were subjected to a pull-down assay with sepharose beads coupled to the RhoA effector protein rhotekin, which specifically binds to only the GTP-bound, activated form. As positive and negative controls for the pull-down assay, we used lysates of the control sample, which was preloaded with either the non-hydrolysable GTPγS (positive control) or GDP (negative control). The eluate fractions from the pull-down were separated by SDS-PAGE and subsequently analyzed by Western blotting with a RhoA specific antibody. The RhoA detected in this case should be exclusively in the GTP-bound form (Fig. 5A, top panel). Band intensities were strongest for the positive control, as expected, and increasing with the time of EBV treatment. The intensities of the scanned bands were quantified and the mock sample set to a one-fold activation. RhoA-GTP levels in the mock sample corresponded to the GDP loaded negative control, while a max. 2.5-fold increase was observed after loading with non-hydrolysable GTP. In the EBV treated samples we observed a step-wise increase with time of treatment, where max levels of approx. 2.4-fold activation were reached after 60 min (Fig.

5B). These results indicated that EBV treatment of AGS cells could indeed trigger activation of RhoA, which results in the previously demonstrated EBV-mediated reorganization of the actin cytoskeleton.

EBV modulates the microtubuli network and increases tubulin acetylation.

By binding to integrin and the subsequent activation of FAK, KSHV leads to rearrangements in the actin cytoskeleton as well as changes in the microtubuli (MT) network, which are facilitating virus entry into the host cell (Sharma-Walia et al. 2004; Naranatt et al. 2005; Raghu et al. 2007). We, therefore, wanted to investigate whether the same was the case for EBV. AGS cells serum-starved for 24h were thus exposed to EBV for either 15 min or 60 min, fixed, permeabilized and reacted with a mouse antibody to α-tubulin (Fig. 6A left panels) or a mouse antibody recognizing actetylated tubulin (Fig. 6A right panels), which is a quantitative indication of changes in MT stabilization (Piperno 1987). Control cells showed a tight meshwork of microtubuli, which was concomitant with evenly distributed low acetylation levels. The microtubuli in the control cells did not have any particular direction or ordering. After 15 min or EBV treatment, the tubuli network appeared less dense, especially around the edges of the cells. The acetylation pattern was substantially stronger around the nucleus and appeared on a filamentous tubuli structure stretching from the nucleus to the edges of the cell. The pattern grew more pronounced after 60 min of EBV exposure, when tubuli appeared as ordered, stretched hair-like structures pointing to the cell edges that had become uneven and pointed in several places. At this point the acetylation was almost exclusively visible around the nucleus.

To further analyze the influence of EBV on MT dynamics we quantified the levels of tubuli acetylation by Western blotting (Fig. 6B). While mock treated cells contained moderate levels of acetylated tubulin, these levels first decreased upon EBV addition and then steadily

increased over time for the first 30 min to decrease after 60 min (Fig. 6B upper panel). The levels of total α-tubulin remained unchanged, thus demonstrating the specificity of tubulin acetylation (Fig. 6B, lower panel). Quantification of the Western blot bands, where levels of mock treated cells were set to one-fold acetylation, showed that after a first drop to around 0.5-fold, acetylation levels increased to 1.7-fold after 30 min and decreased at later time points (Fig. 6C). These results indicated that EBV binding first led to a destabilisation of microtubuli, which resulted in a reorganization and stabilisation later during EBV entry.

Discussion

This is the first study to investigate EBV-mediated intracellular signalling processes associated with EBV entry into epithelial cells.

Based on the recent finding of EBV binding to integrins and their crucial role in infection (Tugizov et al. 2003), we assessed the significance of integrin signalling for EBV entry and analyzed virus triggered processes inside the host cell using a tissue culture system with the gastric epithelial model cell line AGS. The CD21-negative AGS cells had previously been evaluated to be susceptible to EBV (Imai et al. 1998), and we managed to substantially improve the efficiency of cell-free infection using a spinoculation-based method originally developed for the EBV infection of B cells (Dorner et al. 2008).

Our data showed that, apart from serving as an attachment factor binding to EBV BMRF-2 (Tugizov et al. 2003; Xiao et al. 2007; Xiao et al. 2008), β_1 integrins transmitted signals that were crucial for augmenting EBV infection. These signals were most likely triggered by EBV itself and led to FAK activation by autophosphorylation at Y397. We further demonstrated that downstream signalling involving PI3K and c-Src was essential for infection and required for the EBV-induced formation of actin stress fibres and degradation of cortical actin in the epithelial cell. The stress fibre formation was most likely regulated by a proven EBV-dependent activation of the small GTPase RhoA. In addition, we also discovered a rearrangement of microtubuli upon virus binding to cells, which was reflected by a transient change in the stabilization state (summarized in Figure 7). Binding to integrins is a proven concept in the world of herpes viruses, and signalling leads to manifold effects facilitating virus entry, ranging from the disassembly of actin stress fibres enabling faster nuclear trafficking of capsids for HCMV (Wang et al. 2005) to the modulation of microtubule dynamics for DNA delivery of KSHV (Sharma-Walia et al. 2004; Naranatt et al. 2005). Our observations with EBV thus fit the picture; integrins and downstream signalling led to

cytoskeletal changes that were clearly linked with the efficiency of infection. Apart from the inhibition of EBV infection, we noticed that expression of dominant negative integrins disabling downstream signalling led to morphological changes in the cells that stood in sheer contrast to the changes triggered by EBV: Whilst EBV led to spreading of cells, the dominant negative expressing cells were rounded and unable to form protrusions (data not shown). This finding once again underlines the need for spreading and stress fibre formation for infection and clearly links it to integrins.

FAK activation is one of the key events that follow immediately after integrin-ligand interaction (Akula et al. 2002). We now report EBV mediated FAK activation for the first time that is most likely linked to EBV BMRF-2 binding to integrins. Other members of the herpes virus family are also able to trigger FAK such as KSHV (Sharma-Walia et al. 2004) or HSV-1 (Cheshenko et al. 2003; Cheshenko et al. 2007): KSHV triggers FAK by binding to integrins, HSV-1 triggers FAK activation through binding to nectin-1 on apical cell surfaces and an associated calcium release in the cell (Cheshenko et al. 2003; Cheshenko et al. 2007).

The tyrosine kinase inhibitor AG82 was able to substantially reduce EBV infection, indicating that FAK signalling participated in processes crucial for EBV entry. These processes related to the observed cytoskeletal changes induced by the virus, yet they could also be part of other mechanisms essential for EBV entry, since FAK stands at a signalling crossroads. It also has to be considered that inhibition of infection was only observed at a certain concentration, higher concentrations of AG82 reversed the inhibition. This could be due to multiple functions of FAK in EBV entry, but also to the unspecific behaviour of the inhibitor affecting other tyrosine kinases, which may differ at certain concentrations and which may have opposing functions with respect to EBV infection. The observation of FAK-dependent entry of EBV is reminiscent of KSVH- or HSV-1-entry which is facilitated by virus-induced activation of FAK (Cheshenko et al. 2005; Galen et al. 2006). Downstream

signalling of FAK involves PI3Ks and c-Src family proteins (reviewed in Wiesner 2005) and we demonstrated that these kinases were implicated in the observed EBV-mediated changes in the actin cytoskeleton. Inhibition of their function severely affected EBV infection, hinting at a pivotal role in the EBV epithelial cell entry process. It has to be understood, however, that they may be playing a far bigger part than regulating the observed cytoskeletal rearrangements. Both kinases are vital links between integrin and other receptor-mediated signalling with a variety of cellular functions, including endocytosis, and their role in EBV entry infection will need to be examined in detail. It is also not clear in which order the kinases participate in EBV-triggered signalling cascades. In the case of KSHV-mediated cytoskeletal changes Src controls the activation of PI3K after an integrin trigger (Sharma-Walia et al. 2004), Src and PI3K activation are regulated by two different receptors after HCMV binding to host cells (Wang et al. 2005) and even an uncoupling of the two pathways after a common trigger was demonstrated (Bouchard et al. 2007). Since more receptors or attachment factors for EBV glycoproteins other than BMRF-2 potentially exist on epithelial cells (Borza et al. 2004), a more complex triggering of signalling cascades will have to be investigated once these receptors have been identified.

Viruses have evolved a variety of interactions with host cells to facilitate their entry by inducing changes in the cytoskeleton. While modulation of actin can lead to activation of endocytosis pathways or facilitate virus fusion at the plasma membrane (reviewed in Favoreel et al. 2007), capsids of particularly herpes viruses use microtubule-based transport to reach the nucleus and deliver their DNA (reviewed in Smith and Enquist 2002; Dohner et al. 2005). Here we demonstrate both, EBV initiated changes of the actin and microtubule networks. EBV induced a reorganisation from a predominately cortical actin cytoskeleton to pronounced stress fibres. This stays in contrast to the previously reported disassembly of stress fibres induced by other herpesviruses like HCMV (Wang et al. 2005), but was also observed for

KSHV (Sharma-Walia et al. 2004). It has been proposed that EBV fuses directly with the plasma membrane for entering epithelial cells [5]. Thus the observed disassembly of cortical actin may be crucial to overcome the cortical actin barrier for efficient infection. An example from the herpesvirus family for this mechanism is HSV-1. It probably tackles this problem by activation of calcium signalling pathways (Cheshenko et al. 2003), which are well known to induce cortical actin depolymerization (Pollard 2003). Further evidence that this may be the case is provided by our observation in this study that Cytochalasin D treatment substantially increases EBV infection despite or even because of its actin depolymerizing properties. It became clear, however, that EBV affects actin regulatory elements such as the small GTPase RhoA, which is responsible for signal transduction leading to different modulations in the actin network (Etienne-Manneville and Hall 2002).

Microtubules and microfilaments have long been recognized as crucial in controlling the intracellular movements of viruses. Capsids of KSHV and HSV-1 surf on microtubules to reach the nucleus (Sodeik et al. 1997; Naranatt et al. 2005; Raghu et al. 2007) and they may also play a role at later stages of infection to enable directed release of newly formed virions (Arakawa et al. 2007). We observed that EBV triggered a change from a complex tubuli network to a more organized bundle of strings reaching from the nucleus to the edges of the cell. This directed orientation may well hint at a function in transport of EBV capsids from the plasma membrane to the nucleus. Taken together the here presented study reveals how EBV facilitates its own entry into epithelial cells by triggering signalling processes leading to changes in the cytoskeleton. Understanding EBV-mediated signalling may contribute to as yet undiscovered entry pathways of EBV into epithelial cells. EBV triggered integrin signalling may add to the pathogenesis of EBV-associated epithelial cancer and eventually provide us with new approaches to interfere with EBV infection and thus lead to novel ways of prevention and treatment of EBV-associated epithelial cell neoplasia.

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Figure Legends

Fig. 1. EBV infection is dependent on $\beta_1$integrin binding and signalling function. (A) High-speed centrifugation-purified TPA-induced EBV (B95.8 EBfaV-GFP) was incubated with 0, 2 or 5 μg/ml recombinant soluble $\beta_1$integrin for 1h on ice. The treated EBV was subsequently used to infect AGS cells by spinoculation at 37°C. AGS cells were incubated for 48 h before the infection efficiency was analyzed by measuring GFP expression by flow cytometry. (B) AGS cells were nucleofected with plasmids encoding the DN IL2R$\beta_1$integrin and the IL2R control or left untransfected. Nucleofected cells were incubated for 24h and positively sorted for expression of the IL2R epitope with MACS beads coupled to anti-IL2R. Positive cells and untransfected controls were grown for an additional 24h and infected with EBV by spinoculation at 37°C. The efficiency of infection was determined after 48h by measuring GFP expression by flow cytometry. Infection efficiency was expressed as % of untransfected or mock controls, which were assigned a value of 100%. Each bar represents the mean ± SD of two (A) and three (B) experiments conducted in duplicate or triplicate each?, respectively.

Fig. 2. EBV binding leads to activation of FAK, which is required to augment infection. (A) AGS cells were grown on coverslips and starved in serum-free medium (SFM) for 24h. The cells were left in SFM (controls), treated with 40 μg/ml Fibronectin for 1 h or incubated with EBV for 15 min or 60 min at 37°C. All samples were fixed in PFA, permeabilized and stained with mouse anti-phospho-FAK antibodies (Y397) and a secondary Alexa488-coupled anti-mouse reagent. Samples were mounted in anti-fade reagent containing DAPI and analyzed by epifluorescence. White arrow heads indicate accumulations of phosphorylated FAK. (B) Serum-starved AGS cells were left uninduced (control), induced with 40μg/ml Fibronectin for 1 h or with EBV for 15min and 1h at 37°C. Cells were cooled

on ice, lysed in RIPA buffer and cell debris and nuclei were removed. The supernatants were resolved by 10% SDS PAGE, Western blotted and reacted with mouse anti-phospho-FAK (Y397) and rabbit anti-β-actin antibodies as a loading control. Immunoreactive bands were visualized by an enhanced-chemiluminescence reaction. Band intensities for phosphorylated-FAK were normalized according to β-actin expression and quantified. Bars show band intensities for one representative experiment expressed as % of the uninduced control that was assigned a value of 100 %. (C) AGS cells were incubated with 0, 5, 10, or 25 μM AG82 in DMSO for 30 min at 37°C. Subsequently all samples were EBV infected by spinoculation in presence of AG82. Infection was analyzed after 48 h by flow cytometry measurement of GFP expression. Bars represent mean ± SD of three independent experiments.

Fig. 3. PI3K and c-Src signalling augment EBV infection. AGS cells were incubated for 1 h at 37°C with either 50 μg/ml Ly294002, 1μM Wortmannin, or 20μM PP2 and subsequently spinoculated with EBV as in Fig. 1 but in presence of the inhibitor. Infection was analyzed after 48 h by flow cytometry measurement of GFP expression. Bars represent mean ± SD of three independent experiments and are expressed as % of a mock-treated control that was assigned a value of 100%.

Fig. 4. EBV binding induces remodelling of the actin cytoskeleton facilitating infection. (A) AGS cells grown on coverslips were serum starved for 24 h and subsequently left uninduced or treated with either 40 μg/ml Fibronectin for 1h or with EBV for 15 min and 60 min. The cells were fixed in paraformaldehyde, permeabilized and stained with Phalloidin coupled to FITC to visualize actin filaments. Samples were mounted in anti-fade reagent containing DAPI and analyzed by epifluorescence. (B) AGS cells on coverslips were serum starved for 24 h and subsequently treated for 1 h with either 10μM AG82, 50 μg/ml

Ly294002, or 20μM PP2. The cells were then either left uninduced (control) or treated with EBV for 15 min or 60 min in presence of the inhibitors before fixation and Phalloidin-FITC staining as described for (A). (C) AGS cells were treated with 100μM Cytochalasin D (CytD) for 1h and subsequently spinoculated with EBV in presence of fresh Cytochalasin D. Infection was analyzed after 48h by flow cytometry measurement of GFP expression. Bars represent mean ± SD of three independent experiments.

Fig. 5. EBV induces RhoA GTPase in AGS cells. (A) Serum-starved AGS cells were either left untreated (mock) or induced with EBV for 5, 15, 30, or 60 min. The cells were lysed and equal amounts of lysates were used to capture the GTP-bound form of RhoA by affinity precipitation with rhothekin coupled sepharose beads. For positive and negative controls the lysates were loaded with GTPγS and GDP respectively prior to precipitation. The proteins captured by beads were analyzed by 10% SDS PAGE. Gels were blotted, probed with mouse anti-RhoA antibody and visualized by an enhanced chemiluminescence reaction. The bottom panel shows normalized cell lysates for total RhoA as a loading control. (B) Quantitation of EBV-induced RhoA. The bands from panel A were scanned and their intensities quantified after normalization to total RhoA expression. RhoA-GTP levels in mock treated cells were considered one-fold activation for comparison to EBV induced cells. Bars represent band intensities from one representative experiment.

Fig. 6. EBV induces changes in the microtubule organisation of AGS cells. (A) AGS cells grown on coverslips were serum starved for 24 h, left untreated or spinoculated with EBV and incubated for 15 min or 60 min at 37°C. The cells were paraformaldehyde fixed, permeabilized and stained with anti-α-tubulin (left panels) or anti-acetylated-tubulin mouse antibodies and Alexa488 coupled secondary anti-mouse reagent. Samples mounted in anti-

fade containing DAPI were analyzed by epifluorescence. (B) Serum-starved AGS cells were either left untreated (mock) or induced with EBV for 5, 15, 30, or 60 min. The cells were lysed in RIPA buffer and lysates cleared of cell debris and nuclei. Supernatants were separated by 10% SDS PAGE, Western blotted and probed with anti-acetylated-tubulin and anti-α-tubulin as a loading control. Immunoreactive bands were visualized by enhanced-chemiluminescence. (C) Quantitation of tubulin acetylation. The intensities of bands from the upper panel of B were measured and normalized according to total tubulin expression (B lower panel). Tubulin acetylation in EBV treated samples was expressed as fold of mock treated cells. Bars represent values from one representative experiment.

Fig. 7. FAK-PI3K-Src-RhoA-cytoskeleton signalling pathways in epithelial cells induced by EBV. A schematic representation of the EBV-integrin induced hypothetic signal cascade deciphered in this study is shown. Following EBV interactions with integrins through BMRF-2 binding FAK is activated by auto-phosphorylation at Y397, which will create a binding site for SH2 domains of downstream kinases. The downstream kinases involved include PI3K and c-Src, even though their order has not been determined yet. Activated kinases induce activation of RhoA and its transition in a GTP-bound state, which leads to the formation of stress fibres. An as yet unidentified branch of the cascade triggers changes in the microtubuli network. Inhibitors of integrins, FAK, PI3K and c-Src used in this study are indicated in grey and their point of interference is given with T-bars. Solid arrows depict identified pathways, while dashed arrows depict pathways, of which the order has not been dissected in this work. Kinases are boxed in grey.

Figure 1

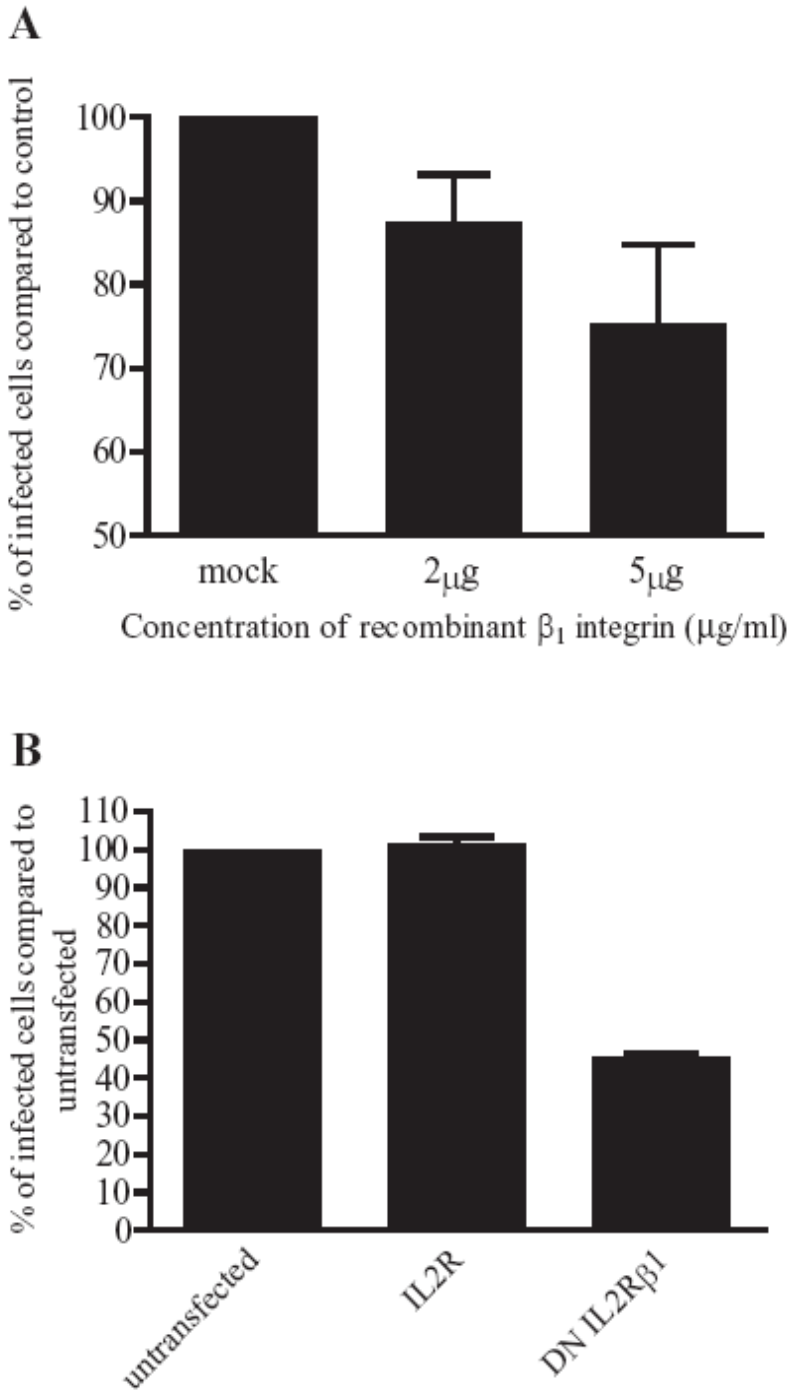

A
% of infected cells compared to control
100
90
80
70
60
50
mock
2μg
5μg
Concentration of recombinant β1 integrin (μg/ml)
B
% of infected cells compared to untransfected
110
100
90
80
70
60
50
40
30
20
10
0
untransfected
IL2R
DN IL2Rβ1

Figure 2

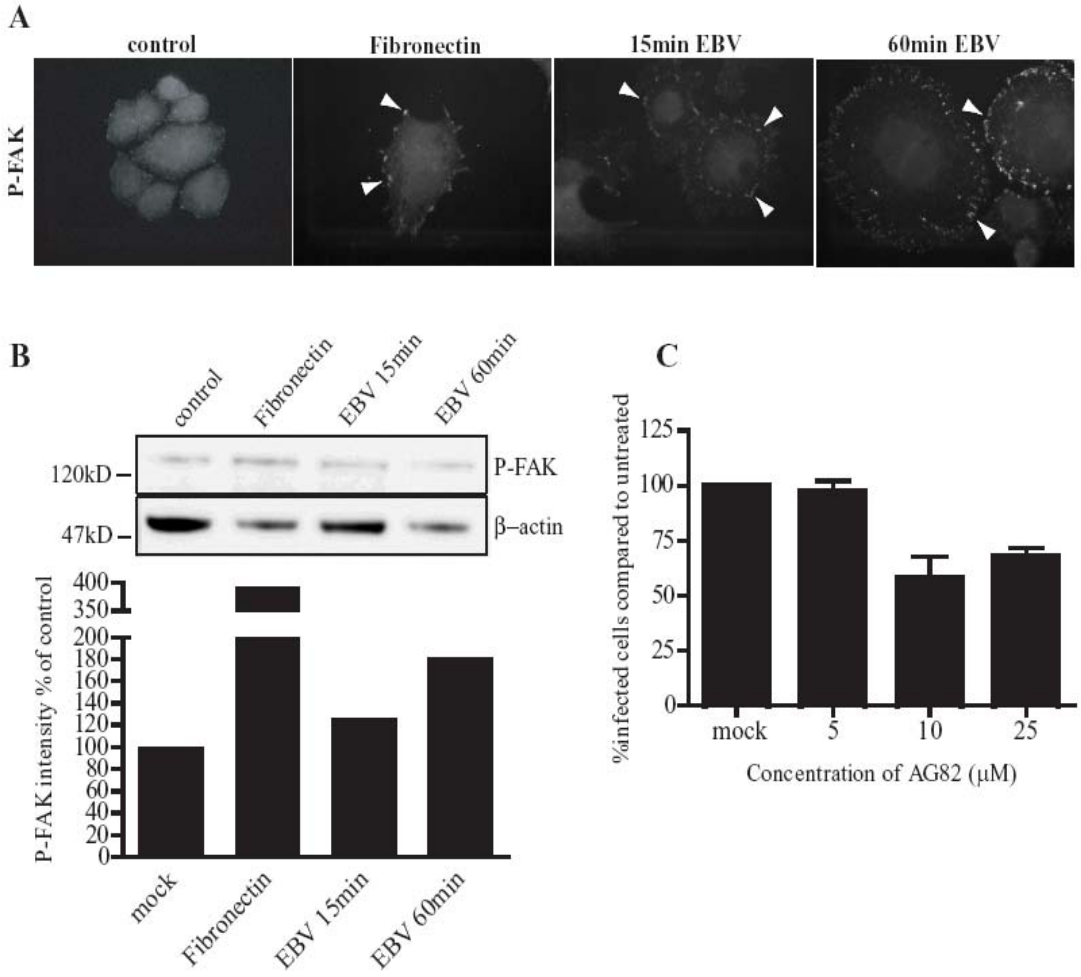

A
control
Fibronectin
15min EBV
60min EBV
P-FAK
B
control
Fibronectin
EBV 15min
EBV 60min
120kD
47kD
P-FAK
β–actin
P-FAK intensity % of control
400
350
200
180
160
140
120
100
80
60
40
20
0
mock
Fibronectin
EBV 15min
EBV 60min
C
%infected cells compared to untreated
125
100
75
50
25
0
mock
5
10
25
Concentration of AG82 (μM)

Figure 3

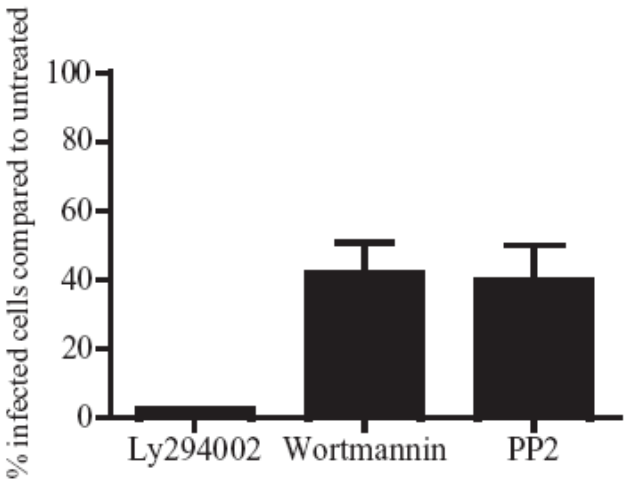

% infected cells compared to untreated
100
80
60
40
20
0
Ly294002
Wortmannin
PP2

Figure 4

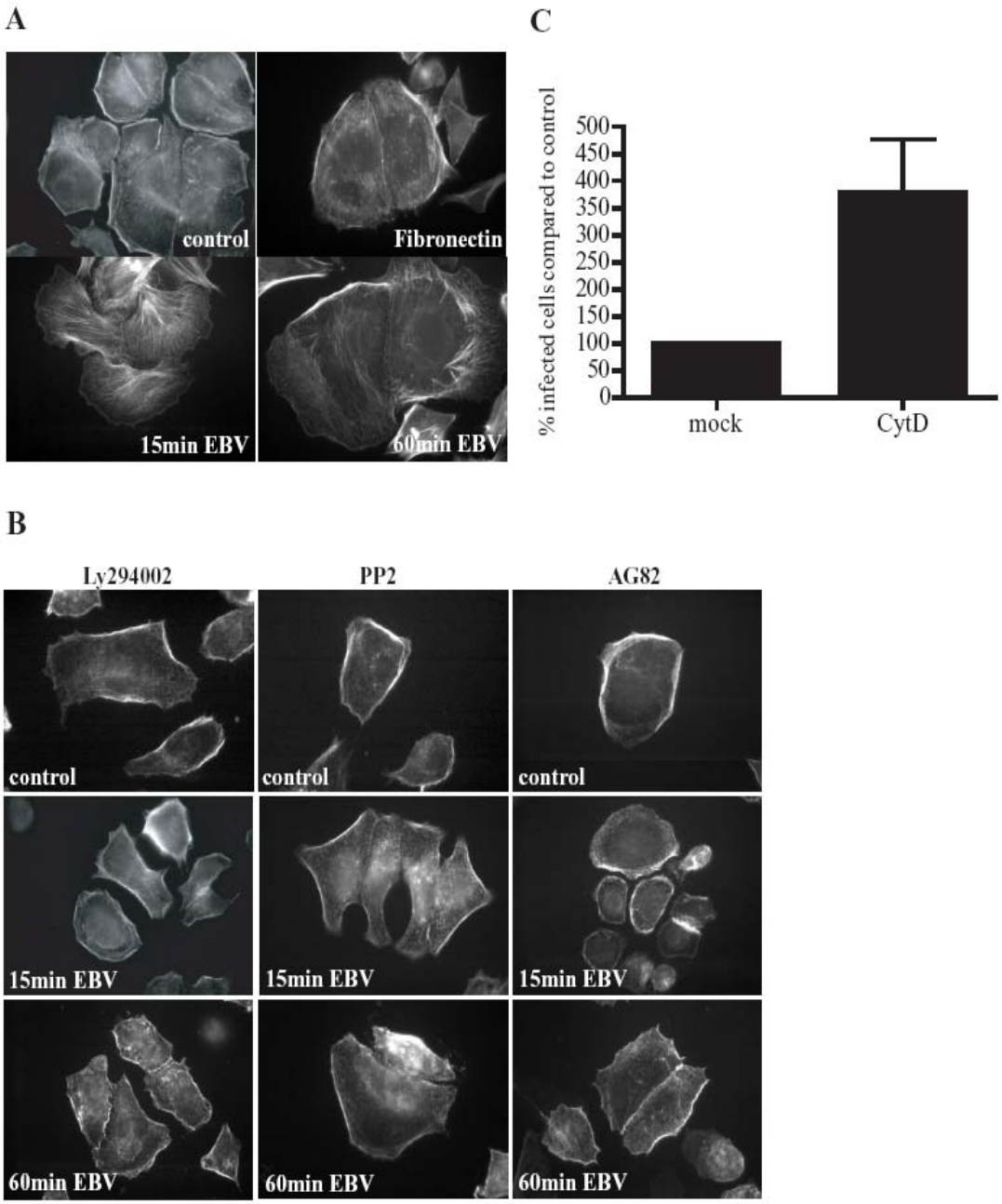

A
control
Fibronectin
15min EBV
60min EBV
C
% infected cells compared to control
500
450
400
350
300
250
200
150
100
50
0
mock
CytD
B
Ly294002
PP2
AG82
control
control
control
15min EBV
15min EBV
15min EBV
60min EBV
60min EBV
60min EBV

Figure 5

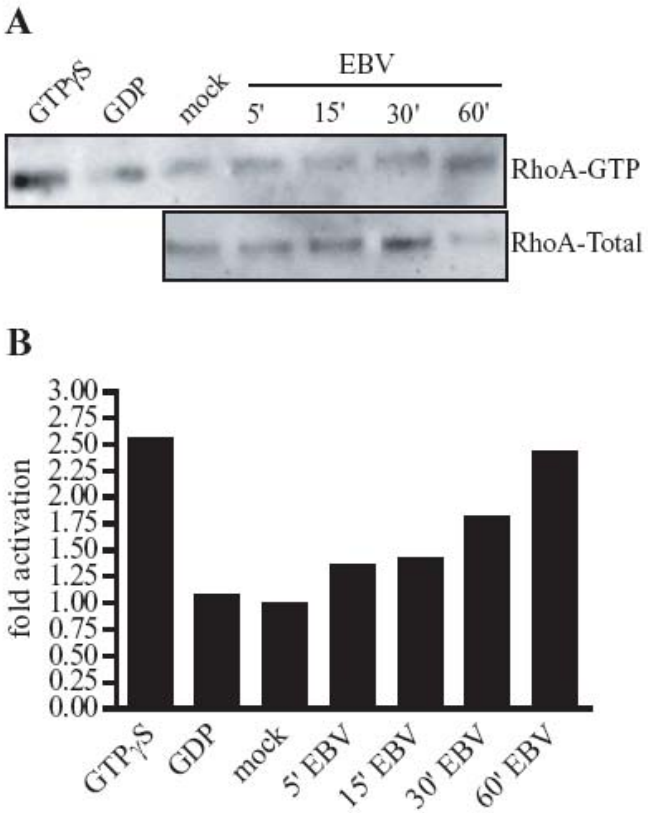

A
GTPγS
GDP
mock
EBV
5'
15'
30'
60'
RhoA-GTP
RhoA-Total
B
fold activation
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
GTPγS
GDP
mock
5' EBV
15' EBV
30' EBV
60' EBV

Figure 6

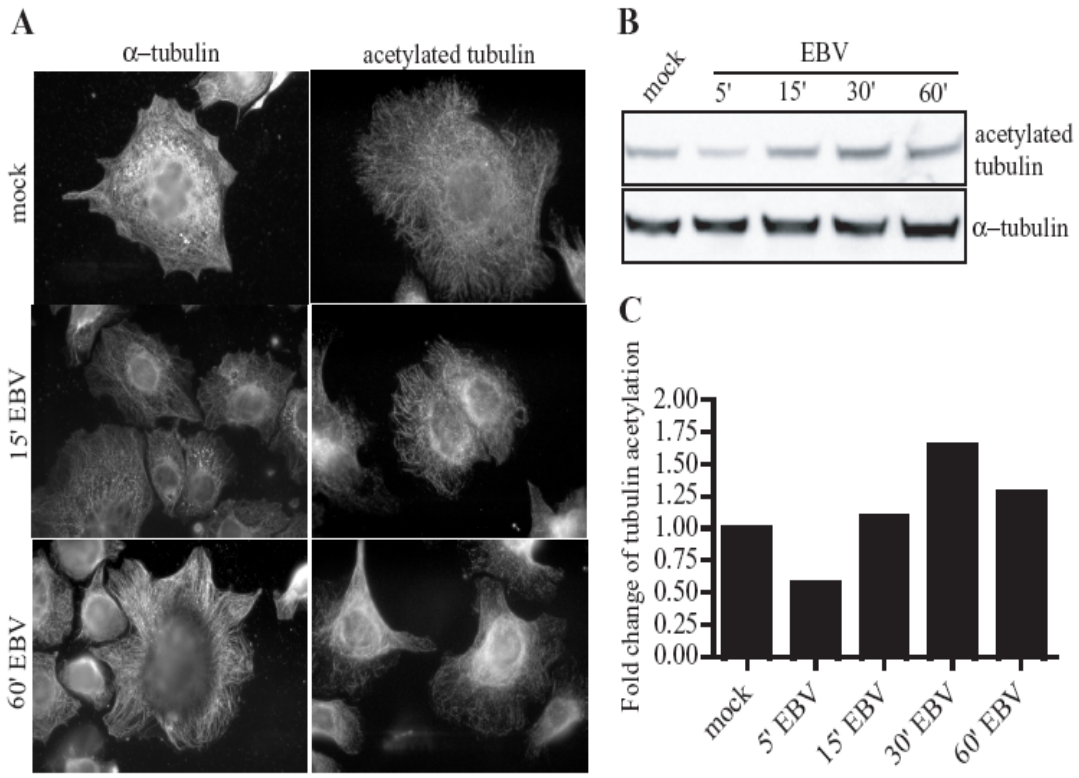
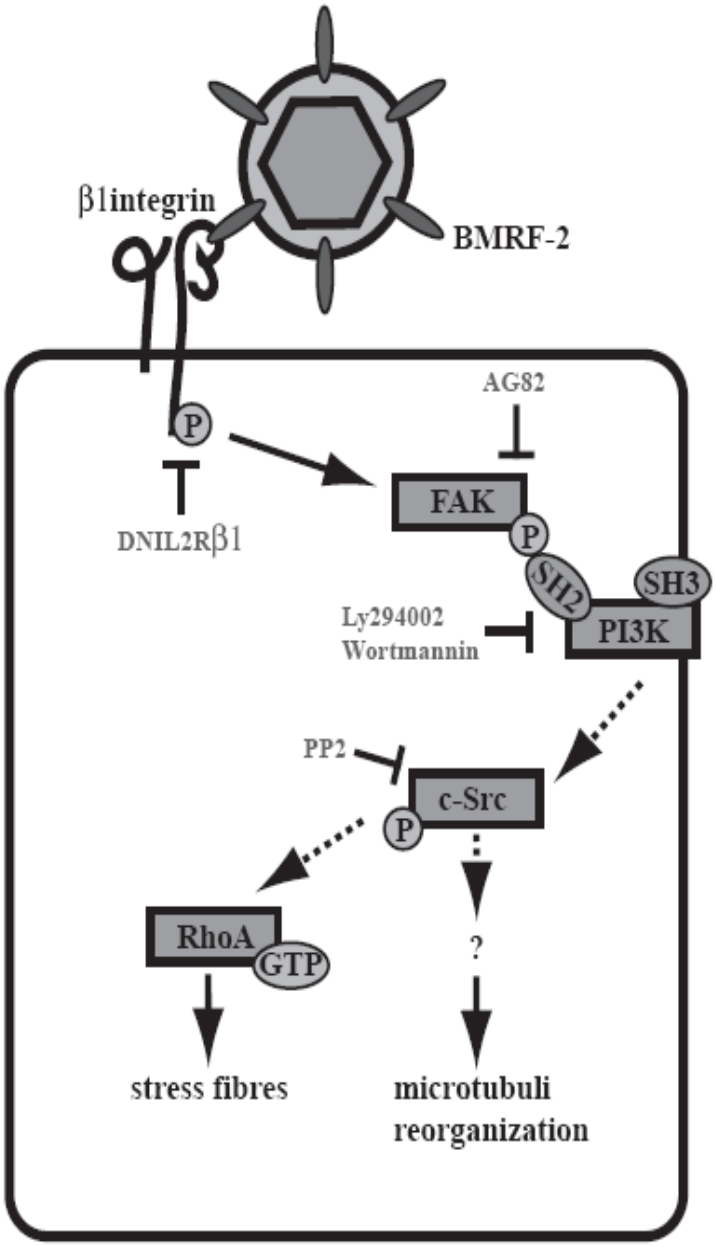

A
α–tubulin
acetylated tubulin
mock
15' EBV
60' EBV
B
mock
EBV
5'
15'
30'
60'
acetylated tubulin
α–tubulin
C
Fold change of tubulin acetylation
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
mock
5' EBV
15' EBV
30' EBV
60' EBV

Figure 7


β1integrin
BMRF-2
AG82
P
DNIL2Rβ1
FAK
P
SH2
SH3
Ly294002
Wortmannin
PI3K
PP2
c-Src
P
RhoA
GTP
?
stress fibres
microtubuli
reorganization

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Immune activation suppresses initiation of lytic Epstein-Barr virus infection

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Summary

Primary infection with Epstein-Barr virus (EBV) is asymptomatic in children with immature immune systems but may manifest as infectious mononucleosis, a vigorous immune activation, in adolescents or adults with mature immune systems. Infectious mononucleosis and chronic immune activation are linked to increased risk for EBV-associated lymphoma. Here we show that EBV initiates progressive lytic infection by expression of *BZLF-1* and the late lytic genes *gp85* and *gp350/220* in cord blood mononuclear cells (CBMC) but not in peripheral blood mononuclear cells (PBMC) from EBV-naive adults after EBV infection *ex vivo*. Lower levels of proinflammatory cytokines in CBMC, used to model a state of minimal immune activation and immature immunity, than in PBMC were associated with lytic EBV infection. Triggering the innate immunity specifically via Toll-like receptor-9 of B cells substantially suppressed *BZLF-1* mRNA expression in acute EBV infection *ex vivo* and in anti-IgG-stimulated chronically latently EBV-infected Akata Burkitt lymphoma cells. This was mediated in part by IL-12 and IFN-γ. These results identify immune activation as critical factor for the suppression of initiation of lytic EBV infection. We hypothesize that immune activation contributes to EBV-associated lymphomagenesis by suppressing lytic EBV and in turn promotes latent EBV with transformation potential.

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Introduction

Epstein-Barr virus (EBV), a human B lymphotropic gammaherpesvirus, infects at least 90% of the world's human population. Different EBV latency gene programs allow EBV to persist in the host in latently infected B cells. Proliferation of the latently infected cells propagates EBV to the daughter cells. Latent EBV may switch to its lytic gene expression program, leading to EBV replication and subsequent lysis of the infected cell (Cohen, 2000; Rickinson and Kieff, 2001; Thorley-Lawson, 2001; Thorley-Lawson and Gross, 2004).

The vast majority of primary EBV infections occur in infants and toddlers and are usually asymptomatic (Biggar *et al.*, 1978; Chan *et al.*, 2001). By contrast, primary EBV infection in adolescence or adulthood may manifest as infectious mononucleosis (IM) (Biggar *et al.*, 1978), with fever and enlargement of tonsils, lymph nodes, liver and spleen. This clinical presentation results from the vigorous immune activation involving proinflammatory cytokines (Foss *et al.*, 1994; Chan *et al.*, 2001; Rickinson and Kieff, 2001).

Epstein-Barr virus is also associated with B cell lymphoproliferative disorders, including Burkitt lymphoma, Hodgkin lymphoma, and post-transplant lymphoproliferative disease harbouring latent EBV. Infection of B cells with EBV *in vitro* in the absence of immune control is associated with B cell proliferation and transformation, indicating the oncogenic potential of EBV (Rickinson and Kieff, 2001). Immunosuppression subsequent to organ transplantation or secondary to infection with the human immunodeficiency virus increases the risk of EBV-associated lymphoproliferation (Cohen, 2000; Rickinson and Kieff, 2001; Thorley-Lawson, 2001). Also immunocompetent patients may develop Burkitt lymphomas and Hodgkin lymphomas harbouring EBV.

Burkitt lymphoma harbouring EBV is mainly seen in areas that are endemic for malaria leading to the speculation that repeated immune activation by chronic malaria or other infections is an important pathogenic factor for this tumour (Rochford *et al.*, 2005). Strikingly, young

adults, experiencing IM and its vigorous immune activation to primary EBV infection, are at increased risk for EBV-positive Hodgkin lymphoma (Hjalgrim *et al.*, 2003). Thus, immune activation seems to be a critical pathogenic factor in EBV-associated lymphomagenesis. The impact of activation via the innate immunity in this process is largely unknown.

Toll-like receptors (TLRs) are key players in the innate immunity. TLRs are transmembrane receptors related to the TOLL protein of *Drosophila* (Hashimoto *et al.*, 1988). They are involved in the recognition of pathogens and microbial products and activate antimicrobial effector pathways (Medzhitov, 2001). Among other TLRs, B cells express TLR-9 (Hornung *et al.*, 2002). TLR-9 sensors unmethylated CpG (cytosine-guanosin) dinucleotides within particular oligodeoxynucleotide sequences of microorganisms as well as the malaria pigment hemozoin (Coban *et al.*, 2005). While triggering TLR-9 increases transformation rates of *ex vivo* EBV-infected B cells (Tragiai *et al.*, 2004), its effect on the EBV gene expression pattern is unknown.

Based on above-mentioned epidemiological and *in vitro*

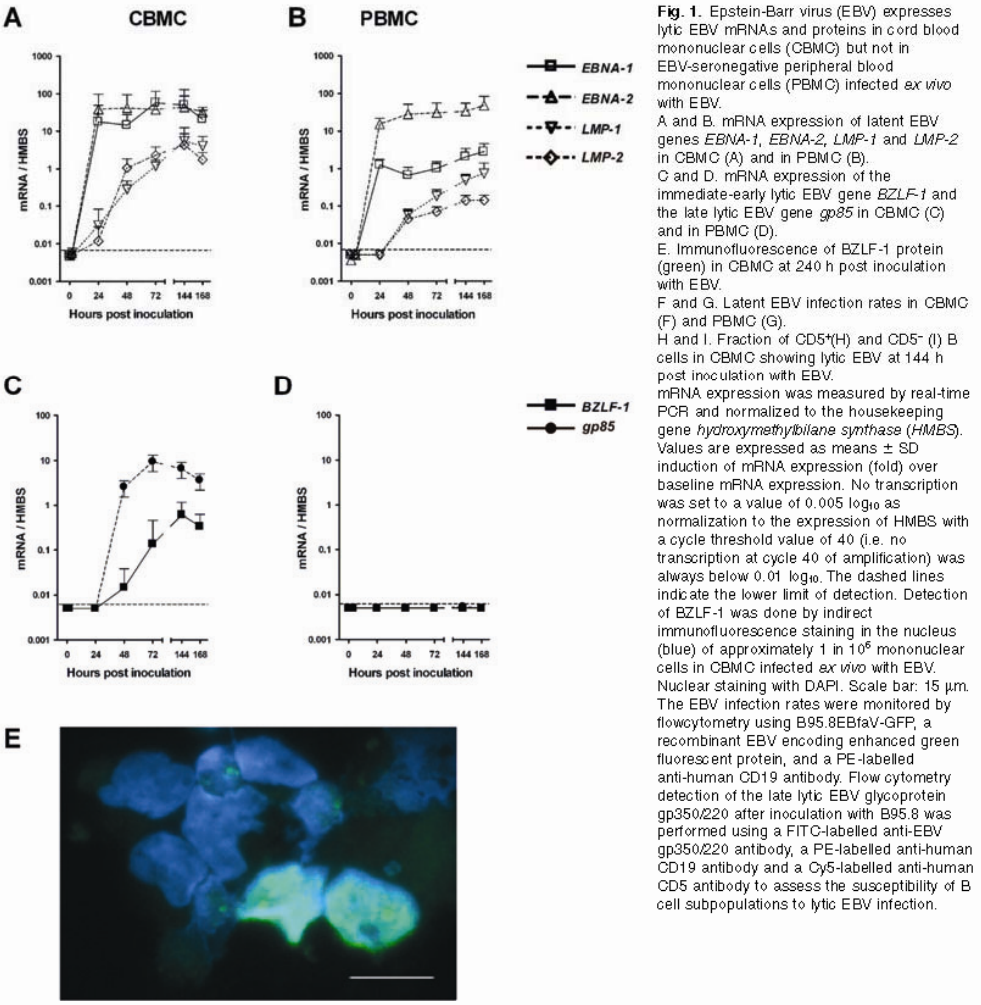

Fig. 1. Epstein-Barr virus (EBV) expresses lytic EBV mRNAs and proteins in cord blood mononuclear cells (CBMC) but not in EBV-seronegative peripheral blood mononuclear cells (PBMC) infected *ex vivo* with EBV.
A and B. mRNA expression of latent EBV genes *EBNA-1*, *EBNA-2*, *LMP-1* and *LMP-2* in CBMC (A) and in PBMC (B).
C and D. mRNA expression of the immediate-early lytic EBV gene *BZLF-1* and the late lytic EBV gene *gp85* in CBMC (C) and in PBMC (D).
E. Immunofluorescence of BZLF-1 protein (green) in CBMC at 240 h post inoculation with EBV.
F and G. Latent EBV infection rates in CBMC (F) and PBMC (G).
H and I. Fraction of $CD5^+$(H) and $CD5^-$ (I) B cells in CBMC showing lytic EBV at 144 h post inoculation with EBV.
mRNA expression was measured by real-time PCR and normalized to the housekeeping gene *hydroxymethylbilane synthase* (*HMBS*). Values are expressed as means ± SD induction of mRNA expression (fold) over baseline mRNA expression. No transcription was set to a value of 0.005 $\log_{10}$ as normalization to the expression of HMBS with a cycle threshold value of 40 (i.e. no transcription at cycle 40 of amplification) was always below 0.01 $\log_{10}$. The dashed lines indicate the lower limit of detection. Detection of BZLF-1 was done by indirect immunofluorescence staining in the nucleus (blue) of approximately 1 in 10^6 mononuclear cells in CBMC infected *ex vivo* with EBV. Nuclear staining with DAPI. Scale bar: 15 µm. The EBV infection rates were monitored by flowcytometry using B95.8EBfaV-GFP, a recombinant EBV encoding enhanced green fluorescent protein, and a PE-labelled anti-human CD19 antibody. Flow cytometry detection of the late lytic EBV glycoprotein gp350/220 after inoculation with B95.8 was performed using a FITC-labelled anti-EBV gp350/220 antibody, a PE-labelled anti-human CD19 antibody and a Cy5-labelled anti-human CD5 antibody to assess the susceptibility of B cell subpopulations to lytic EBV infection.

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observations, we hypothesized that immune activation affects EBV gene expression. We tested our hypothesis by activating cord blood mononuclear cells (CBMC) and peripheral blood mononuclear cells (PBMC) from adults acutely infected *ex vivo* with EBV, and chronically EBV-infected Akata Burkitt lymphoma cells. The rationale to use CBMC was its minimal immune activation and maturity status compared with PBMC from adults (Bradley and Cairo, 2005). We avoided bias from pre-existing EBV-specific T-cell immunity by using primary cells only from EBV-naive donors.

Results

Epstein-Barr virus expresses BZLF-1 and gp85 in CBMC, but not in PBMC, after EBV infection ex vivo

We hypothesized that CBMC and adult PBMC, given their different degrees of immune activation and maturation, display distinct EBV gene expression patterns after EBV infection. Using flow cytometry, we first verified that CBMC show a lower degree of immune activation than adult PBMC by assessing the proportion of $CD4^+$ and $CD8^+$ cells expressing HLA-DR. Indeed, in CBMC ($n = 10$ donors), the percentages of $CD4^+$/HLA-DR^+ and $CD8^+$/HLA-DR^+ cells were 0.8 ± 0.5 and 0.8 ± 0.2, respectively, and in PBMC ($n = 4$ donors), they were 11.5 ± 0.4 and 20.5 ± 3.6 respectively. Next, to test our hypothesis, we quantified latent (*EBNA-1*, *EBNA-2*, *LMP-1* and *LMP-2*) and lytic (*BZLF-1*, the initiator of EBV lytic infection, and *gp85*, a late lytic gene) EBV gene mRNA expression in CBMC and adult PBMC after infection with EBV *ex vivo*. We used CBMC to model a state of immature and less vigorous immune responses than in adolescents or adults, and we used PBMC from EBV-naive individuals to prevent potential influences on EBV gene mRNA expression by pre-existing EBV-specific immunity. We measured EBV gene mRNA expression levels by real-time polymerase chain reaction (PCR) and normalized to levels of the housekeeping gene *hydroxymethylbilane synthase (HMBS)* at 0, 2, 24, 48, 72, 144 and 168 h after *in vitro* EBV inoculation of CBMC or PBMC. Similar levels of the latent genes *EBNA-1*, *EBNA-2*, *LMP-1* and *LMP-2* were detected 24 h after EBV inoculation of CBMC or PBMC (Fig. 1A and B), documenting successful infection with EBV. Levels of *EBNA-2* mRNA tended to be higher than those of *EBNA-1*, and mRNA levels of these two genes were higher than those of *LMP-1*, and *LMP-2* both in CBMC and PBMC. By contrast, significant mRNA expression of the lytic genes *BZLF-1* and *gp85* was consistently observed in CBMC (Fig. 1C) at and after 72 h post inoculation of EBV, but was never seen in adult PBMC (Fig. 1D). Accordingly, *BZLF-1* protein was detected by immunofluorescence in CBMC (Fig. 1E), but not in PBMC (not shown). It is known that EBV lytic cycle coincides with host shutoff mediated through

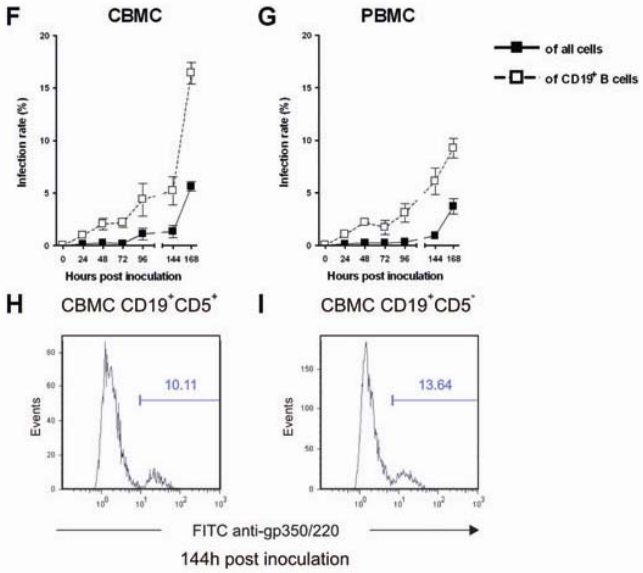

Fig. 1. *cont.*

mRNA degradation (Glaunsinger and Ganem, 2006). If that concerns the housekeeping gene *HMBS*, we used to normalize our data, this could lead to an overestimation of the lytic EBV genes. We found that the cycle threshold (Ct) values for *HMBS* mRNA expression in CBMC ($n = 6$) following *ex vivo* infection with EBV were rather constant in the first 72 h and showed a slight decrease thereafter (not shown), indicating that the abundance of *HMBS* mRNA expression is not diminishing but rather increasing.

The fractions of B cells latently infected with EBV in CBMC and PBMC following ex vivo *infection are similar, and CD5$^+$ and CD5$^-$ B cell subsets in CBMC are equally susceptible to lytic EBV*

We asked whether the difference in lytic EBV gene expression between CBMC and PBMC was due to different EBV infection rates. Thus, we estimated the fractions of latently EBV-infected B cells following *ex vivo* infection using an enhanced green fluorescent protein expressing B95.8 EBV, EBfaV-GFP (Speck and Longnecker, 1999). In separate experiments we documented that *ex vivo* infection with EBfaV-GFP resulted in qualitative and quantitative latent EBV gene expression patterns in CBMC and PBMC similar to those observed following *ex vivo* infection with B95.8 (M. Dorner *et al.*, manuscript in preparation), suggesting that EBfaV-GFP is a valid substitute of B95.8. The fraction of CD19+ B cells in CBMC ($n = 3$) and PBMC ($n = 3$) at baseline was 11.6 ± 2.4% and 7.3 ± 2.3% respectively. The overall EBV infection rates were similar in CBMC and PBMC in the first 144 h after EBV inoculation *ex vivo* when they reached around 1% in relation to all cells and around 4–5% of CD19$^+$ B cells (Fig. 1F and G). To assess the fractions of lytically infected cells we stained the cells for the late lytic glycoprotein gp350/220 which is expressed on the plasma membrane (Gong and Kieff, 1990) following *ex vivo* infection with 95.8 EBV. Using flow cytometry we documented that the proportion of B cells exhibiting lytic EBV infection peaked between 2 and 3% at 144 h post EBV inoculation in CBMC while no cells expressing lytic EBV were found in PBMC (not shown). The vast majority of B cells in CBMC ($n = 3$) belonged to the CD5$^+$ B cell subset (74.17 ± 8.15%), whereas in adult PBMC the minority of B cells were CD5$^+$ (28.81 ± 11.16%). To evaluate whether the susceptibility of these B cell subsets in CBMC to lytic EBV is different, we determined the numbers of CD5$^+$ and CD5$^-$ B cells staining for gp350/220. Flow cytometry showed that the numbers of CD5$^+$ B cells in CBMC ($n = 3$) were 78 ± 5% and that the proportions of CD5$^+$ and CD5$^-$ B cells expressing gp 350/220 in CBMC ($n = 3$) were similar (11.5 ± 3.2% vs. 12.8 ± 3.8%) at 144 h following *ex vivo* infection (Fig. 1H and I), indicating comparable susceptibility to EBV lytic infection. Thus, although the fractions of B cells infected with EBV in CBMC and PBMC following *ex vivo* infection were similar, CBMC exhibited lytic EBV infection whereas PBMC did not. The expression of gp350/220 clearly indicates that the lytic infection is not only initiated (Laichalk and Thorley-Lawson, 2005) but is also fully executed in CBMC. Furthermore, the difference between CBMC and PBMC in lytic EBV gene expression cannot be attributed to the higher content of CD5$^+$ cells in CBMC than in PBMC, because CD5$^+$ and CD5$^-$ cells were equally susceptible to lytic EBV infection.

Cord blood mononuclear cells express lower levels of IL-12 p35, IFN-γ and IL-2 mRNA than PBMC at baseline and in response to EBV

Because CBMC and adult PBMC represent immune cells with dissimilar states of immune maturation with different abilities to express cytokines, we asked whether the distinct EBV gene expression in CBMC and PBMC was associated with differing cytokine gene expression. We compared mRNA levels of proinflammatory cytokines in CBMC and PBMC before and after EBV infection *in vitro*. CBMC displayed lower mRNA levels of *IL-12 p35* ($P = 0.0001$), *IFN-γ* ($P < 0.0075$) and *IL-2* ($P < 0.001$) than PBMC at baseline (Fig. 2). mRNA levels of *TNF-α*, *IL-1β*, *IL-6* and *IL-8* did not significantly differ between CBMC and PBMC at baseline (not shown).

Inoculation with EBV led to increased mRNA levels of *IL-12 p35*, *IFN-γ* and *IL-2* in CBMC and adult PBMC. However, mRNA levels in CBMC never reached the levels observed in adult PBMC, and significant differences between CBMC and adult PBMC were also found after inoculation with EBV (Fig. 3). Because host shutoff mediated through mRNA degradation during EBV lytic gene expression may result in false high positive cytokine mRNA levels, we also measured IL-12, IFN-γ and IL-2 at the protein level. At 96 h after inoculation with EBV, protein levels of IL-12 p40, IFN-γ and IL-2 were 11.4 ± 1.5 pg ml^{-1}, 6.5 ± 2.1 pg ml^{-1} and 0.8 ± 0.6 pg ml^{-1}, respectively, in CBMC supernatants ($n = 4$) versus 22.5 ± 2.3 pg ml^{-1}, 34.5 ± 7.8 pg ml^{-1} and 7.6 ± 1.7 pg ml^{-1}, respectively, in PBMC supernatants ($n = 4$). Overall, mRNA levels of *IL-12 p35*, *IFN-γ* and *IL-2* were lower in CBMC than adult PBMC before EBV infection *ex vivo* and both mRNA and protein levels of these cytokines were also lower in CBMC than adult PBMC after EBV infection *ex vivo*, suggesting that differences in expression levels of these proinflammatory cytokines may be due to maturational differences in cytokine responses and the degree of immune activation or maturation may influence the initiation of lytic EBV infection.

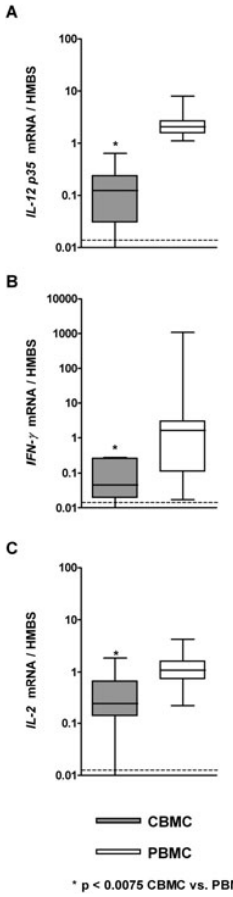

Fig. 2. CBMC express significantly lower mRNA levels of proinflammatory cytokine genes than PBMC at baseline.
A. IL-12 p35.
B. IFN-γ.
C. IL-2.
CBMC ($n = 19$) and PBMC ($n = 20$) were isolated by density-gradient centrifugation. RNA was extracted from cell pellets and treated with DNase to remove residual genomic DNA. The mRNA was reverse transcribed into cDNA using an oligo-d(T)$_{15}$ primer. mRNA expression was measured by real-time PCR and normalized to the housekeeping gene HMBS. Median values (solid black line) of fold mRNA expression in relation to HMBS are shown as box plots with whiskers that extend to the highest and lowest values above and below the box. The dashed lines indicate the lower limit of detection. * refers to CBMC versus PBMC.

The lower levels of IL-12, IFN-γ and IL-2 in CBMC than in PBMC in response to EBV are not associated with higher levels of TGF-β *or* IL-10 *mRNA*

To explore whether lower levels of proinflammatory cytokines in CBMC than PBMC after infection with EBV were associated with higher mRNA levels of anti-inflammatory cytokines in CBMC than in PBMC, we measured mRNA expression of the anti-inflammatory cytokine genes *TGF-β* and *IL-10*. Importantly, the assay we used to detect human *IL-10* is highly host specific and does not detect EBV-encoded viral *IL-10*. At baseline, mRNA levels of *TGF-β* were lower whereas levels of *IL-10* were higher in CBMC than in PBMC (Fig. 4). Following infection with EBV, levels of *TGF-β* remained lower in CBMC compared with in PBMC. By contrast, levels of *IL-10* became significantly lower in CBMC than in PBMC after EBV inoculation *in vitro* (Fig. 4). These findings strongly indicate that the lower levels of induction of IL-12, IFN-γ and IL-2 in CBMC than in PBMC after inoculation with EBV are not due to higher expression of *TGF-β* or *IL-10* mRNA in CBMC than in PBMC.

To investigate the effects of IL-10 on lytic EBV infection, we treated CBMC ($n = 3$) with recombinant human IL-10 at 1, 10, or 100 pg ml^{-1}. Adding IL-10 did not result in significant changes in *BZLF-1* mRNA expression following EBV infection *ex vivo* (not shown). Similarly, neutralizing IL-10 in PBMC from EBV-seronegative adults ($n = 3$) with anti-human IL-10 antibody at 1.0 U ml^{-1} did not result in *BZLF-1* mRNA expression following EBV infection *ex vivo* (not shown). Thus, *IL-10* levels appear to have no effect on EBV lytic gene expression patterns.

rIL-12 and rIFN-γ decrease BZLF-1 *mRNA expression in CBMC during EBV infection* in vitro

Next, we asked whether IL-12 or IFN-γ have an effect on *BZLF-1* expression in CBMC after infection with EBV. We infected CBMC with EBV *ex vivo*, treated with rIL-12, rIFN-γ or both, and measured mRNA expression 96 h after infection when *BZLF-1* is detectable in all of the EBV-infected untreated CBMC cultures (Fig. 5). In CBMC treated with rIL-12 simultaneously with EBV inoculation and then every 24 h, *IL-12 p35* mRNA expression was unchanged, whereas *IFN-γ* mRNA expression increased about 6.5-fold at 96 h, and *BZLF-1* mRNA expression level was reduced by 50%, compared with untreated CBMC (Fig. 5). As expected, treatment of CBMC with rIL-12 also increased the IFN-γ protein concentration in the cell-free supernatant of CBMC compared with no treatment (1368 vs. 37 pg ml^{-1}). This higher increase in protein concentration compared with the increase in mRNA expression may be explained by either accumulation of transcribed protein in the super-

natant or expressed mRNA being transcribed to protein at higher rates, or both. Treatment of CBMC with rIFN-γ, simultaneously with EBV inoculation and then every 24 h, did not change *IFN-γ* mRNA expression and did not influence *IL-12 p35* mRNA expression, but reduced *BZLF-1* mRNA expression by 50% compared with untreated CBMC (Fig. 5). Finally, treatment with both rIL-12 and rIFN-γ simultaneously with EBV inoculation and then every 24 h, did not change *IL-12 p35* mRNA expression, increased *IFN-γ* mRNA expression around 17-fold, and resulted in a stronger suppression (sixfold) of *BZLF-1* mRNA expression than when treatment included only one of both cytokines (Fig. 5). Conversely, we treated adult PBMC infected with EBV *ex vivo* with antibodies to IL-12 and IFN-γ and could not provoke *BZLF-1* mRNA expression (not shown). Thus, substitution of the proinflammatory cytokines IL-12 and IFN-γ partially suppressed *BZLF-1* mRNA expression in CBMC infected with EBV *ex vivo*, indicating that the weaker proinflammatory immune response in CBMC contributes to the initiation of lytic EBV infection seen in CBMC. The failure to provoke *BZLF1* mRNA expression in acutely infected PBMC with antibodies to IL-12 and IFN-γ together with their incomplete suppression of *BZLF-1* mRNA expression suggests that these two cytokines are

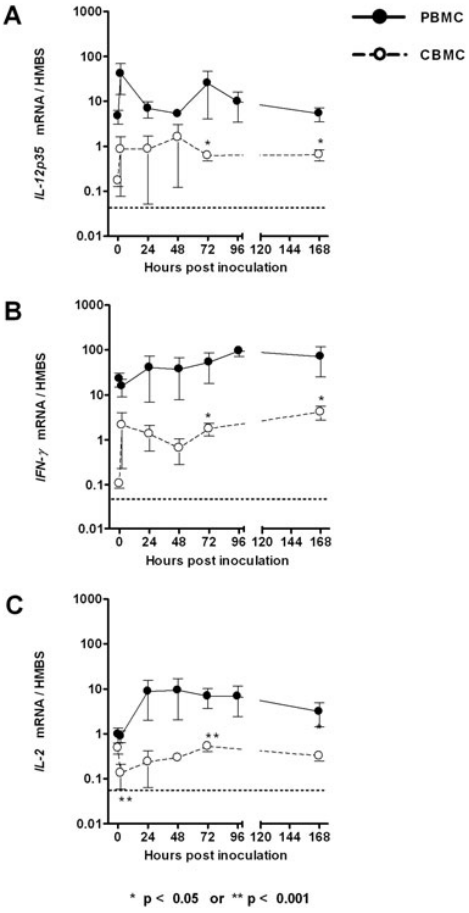

Fig. 3. CBMC express significantly lower mRNA levels of proinflammatory cytokine genes than PBMC from EBV-seronegative adults following infection with EBV *ex vivo*.
A. IL-12 p35.
B. IFN-γ.
C. *IL-2*.
mRNA expression was measured by real-time PCR and normalized to the housekeeping gene HMBS. Results are means ± SD of mRNA expression normalized to HMBS (fold) during 7 days of culture. The dashed lines indicate the lower limit of detection. * or ** refers to CBMC ($n = 3$–8) versus PBMC ($n = 3$).

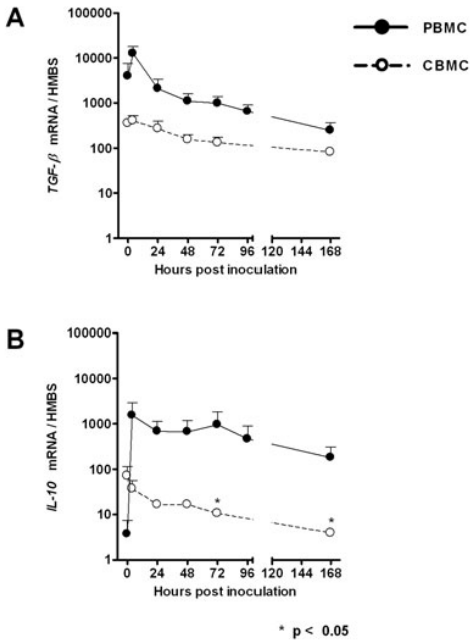

Fig. 4. CBMC express lower mRNA levels of anti-inflammatory cytokine genes than PBMC from EBV-seronegative adults in response *ex vivo* infection with EBV.
A. TGF-β.
B. IL-10.
mRNA expression was measured by real-time PCR and normalized to the housekeeping gene HMBS. Results are means ± SD of mRNA expression normalized to HMBS (fold) during 7 days of culture. * refers to CBMC ($n = 3$–8) versus PBMC ($n = 3$).

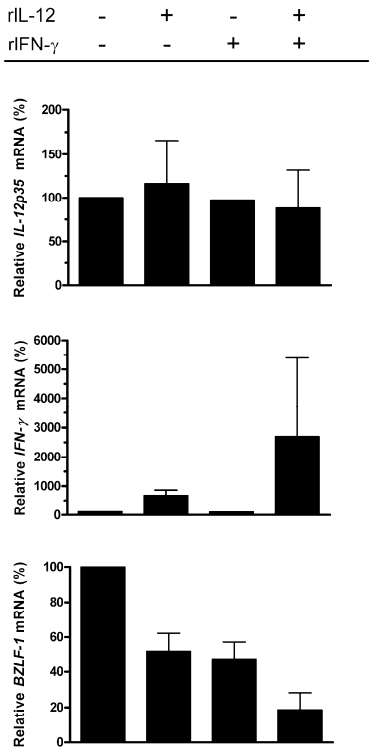

Fig. 5. rIL-12, rIFN-γ, or both suppress the transcription of *BZLF-1* in CBMC infected with EBV *ex vivo*. rIL-12, rIFN-γ, or both were added together with EBV and then every 24 h over 96 h to the cultures. mRNA was measured by real-time PCR. Results shown are from one representative experiment of six from CBMC from different donors. RNA was extracted and analysed from four different cell pellets per condition, except for the treatments with rIFN-γ (two cell pellets per condition). Means ± SD represent the differences of mRNA expression between treated and untreated samples after normalization to the housekeeping gene HMBS.

not the only players suppressing the initiation of lytic EBV infection.

CpG ODN 2006 suppress BZLF-1 *mRNA expression in CBMC infected with EBV* in vitro

We next sought to test whether other means of immune stimulation would lead to suppression of *BZLF-1* expression in CBMC. To stimulate CBMC we used the unmethylated CpG-containing ODN 2006 that triggers the innate pathogen-associated molecular pattern recognition receptor TLR-9 which is also expressed in B cells (Hornung *et al.*, 2002) and exerts a proinflammatory effect (Peng, 2005). We asked if stimulating CBMC with CpG ODN 2006 alters EBV gene expression. Thus, we cultured CBMC with or without CpG ODN 2006 and with or without EBV for 96 h respectively. Treatment of uninfected CBMC with CpG ODN 2006 resulted in a 2.6-fold increase of *TLR-9* mRNA expression versus no treatment (Fig. 6A). EBV infection by itself led to 3.7-fold increase in levels of *TLR-9* mRNA expression in CBMC over uninfected CBMC (Fig. 6B). The large number of CpG motifs in the EBV DNA genome or a CpG-motif-independent mechanism may explain the upregulation of *TLR-9* by EBV. Treatment of EBV-inoculated CBMC with CpG ODN 2006 resulted in a further but not significant increase of *TLR-9* mRNA expression (Fig. 6B). As expected, inoculation of CBMC with EBV resulted in marked expression of *BZLF-1* mRNA. By contrast, EBV-infected CBMC cultures treated with CpG ODN 2006 exhibited a 5.8-fold lower *BZLF-1* mRNA expression (Fig. 6C). Although EBV itself induced *TLR-9* mRNA expression in CBMC, the induction did not suppress *BZLF-1* mRNA expression. Therefore, the reduction of *BZLF-1* mRNA expression in EBV-infected CBMC after CpG ODN 2006 treatment did not seem to depend on induction of TLR-9, but rather on the additional stimulation by CpG ODN 2006 (e.g. increased TLR-9 signalling mediated by CpG binding). Expression levels of latent EBV gene mRNAs were not significantly different in untreated or CpG ODN 2006-treated EBV-infected CBMC (not shown).

Next, we asked whether triggering of other TLRs present on B cells also results in suppression of lytic EBV. Triggering of TLR-1/2, TLR-4, or TLR-7/8 on EBV-infected CBMC did not result in significant suppression of *BZLF-1* and *gp85* mRNA expression and gp350/220 expression compared with controls (Fig. 6D–F). These data suggest that CpG ODN 2006 stimulation of TLR-9 on EBV-infected CBMC is rather specific in inhibiting the mRNA and protein expression of EBV genes involved in lytic infection but has no effect on latent EBV gene mRNA expression. This suppression of the initiation and completion of lytic EBV infection in turn may support maintenance of EBV latency.

Antibodies to IL-12 and IFN-γ partially restore BZLF-1 *mRNA expression in EBV-infected CBMC treated with CpG ODN 2006*

We next explored whether IL-12 and IFN-γ contribute to the suppression of *BZLF-1* mRNA expression induced after TLR-9 triggering by CpG ODN 2006. We added antibodies to IL-12 and IFN-γ to cultures of CBMC inoculated with EBV and treated with CpG ODN 2006. Indeed, treatment with anti-IL-12 and anti-IFN-γ partially restored expression of *BZLF-1* mRNA in these CBMC

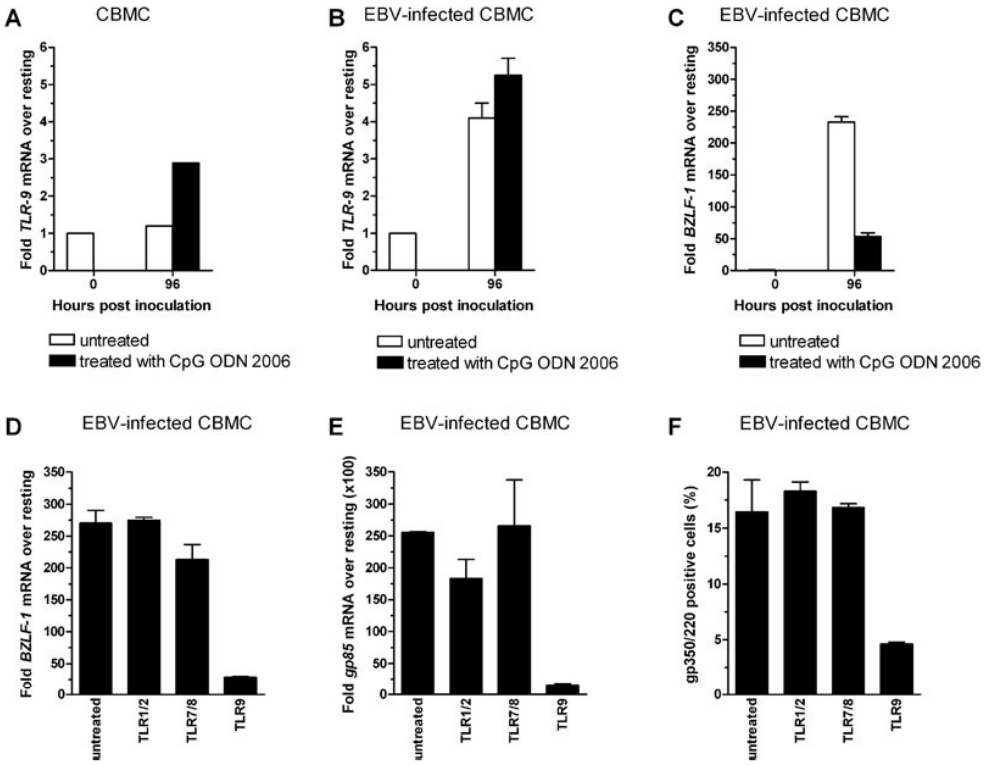

Fig. 6. CpG ODN 2006 specifically suppresses initiation and execution of lytic EBV in CBMC following *ex vivo* infection.
A. mRNA expression of *TLR-9* in CBMC ($n = 3$) treated or not with CpG ODN 2006.
B. mRNA expression of *TLR-9* in CBMC ($n = 3$) infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
C. mRNA expression of *BZLF-1* in CBMC ($n = 3$) infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
D and E. mRNA expression of *BZLF-1* (D) and EBV *glycoprotein (gp) 85* (E) in CBMC ($n = 3$) that were stimulated with ligands of TLRs present in B cells and infected *ex vivo*.
F. Expression of the lytic EBV glycoprotein gp350/220 in CBMC treated with ligands of TLRs present in B cells and infected *ex vivo*.
TLR ligands were added at 0 h and 90 h to 2×10^6 CBMC ($n = 3$) infected *ex vivo* with EBV. Cells were collected at 96 h. Concentrations of TLR ligands were 10 μg ml^{-1} for peptidoglycan (TLR-1/2), 3 μM for R-848 (TLR-7/8) and 1 μM for CpG ODN 2006 (TLR-9). RNA was extracted from two cell pellets per condition, treated with DNase, and reverse-transcribed into cDNA with an oligo-dT15 primer. mRNA expression was measured in duplicate by real-time PCR. Means ± SD of fold induction over resting normalized to the housekeeping gene HMBS.
Flowcytometry was performed using a FITC-anti-EBV gp350/220 antibody. Events shown are gated for $CD19^+$ B cells. One representative experiment of three is shown.

(Fig. 7A). Even though these antibodies exhibited little effect on CpG ODN 2006-induced enhanced *IL-12 p35* and *IFN-γ* mRNA expression (Fig. 7B and D), the protein levels of both cytokines were below the lower limit of detection (Fig. 7C and E). These data at the protein level excluded potentially misleading results due to host shutoff mediated through mRNA degradation during EBV lytic gene expression. Thus, our observations provide evidence that part of the negative impact on the initiation of lytic EBV infection in CBMC exhibited by CpG ODN 2006 through TLR-9 triggering is mediated by IL-12 and IFN-γ.

A key question is which cells are implicated in the effects observed. Thus, we infected highly purified B cells from CBMC and PBMC with EBV. Indeed, *BZLF-1* was expressed in B cells from CBMC but not from PBMC. mRNA and protein levels for IL-12 and IFN-γ were strikingly lower in B cells from CBMC than from PBMC (Fig. 7F–O). Moreover, we could reproduce the inhibitory effects on *BZFL-1* expression when triggering TLR-9

similar as outlined above. TLR-9 triggering was associated with an increase in IL-12 and IFN-γ at the mRNA as well as protein level. Thus, the effects we observed when triggering TLR-9 are rather direct than indirect.

CpG ODN 2006 suppresses induction of BZLF-1 *mRNA expression in Akata Burkitt lymphoma cells*

The above experiments addressed the effect of immune stimulation triggered by cytokines and TLR-9 on the initiation of lytic EBV infection in cells exposed to acute infection with EBV, but not in cells with chronic latent EBV infection. Switching from latent to lytic EBV infection may occur spontaneously or be provoked in EBV-transformed cells by several agents *in vitro* (Kieff and Rickinson, 2001). Cells from the Burkitt lymphoma cell line Akata can readily be provoked to switch from latent to lytic EBV infection within hours by cross-linking their surface IgG using anti-IgG antibodies. Thus, we asked whether triggering of TLR-9 exhibits an effect on the induction of lytic EBV infection in Akata cells, used as a surrogate for Burkitt lymphoma cells. We first determined whether Akata cells express TLR-9. Using quantitative PCR, we demonstrated that Akata cells constitutively express *TLR-9* mRNA. Stimulation of Akata cells with CpG ODN 2006 did not increase *TLR-9* mRNA expression (Fig. 8A). This suggested that TLR-9 expression in the fully differentiated Akata cells was maximal before treatment with CpG ODN 2006 as opposed to CBMC which contain naive B cells and showed an increase in *TLR-9* mRNA expression upon stimulation with CpG ODN 2006 (Fig. 6A). As expected, cross-linking of surface IgG after treatment with anti-IgG provoked the expression of *BZLF-1* mRNA and thus the initiation of lytic EBV infection (Fig. 8B). Treatment of Akata cells with CpG ODN 2006 before treatment with anti-IgG reduced *BZLF-1* mRNA expression provoked by surface IgG cross-linking by 50% (Fig. 8B). By contrast, treatment with CpG ODN 2006 simultaneously or deferred to anti-IgG treatment had no significant effect on the initiation of lytic EBV infection (not shown); indicating that the signalling cascade initiated by anti-IgG appears to be dominant to the intracellular changes subsequent to triggering TLR-9. These data suggest that triggering innate immunity via TLR-9 suppresses the initiation of lytic EBV infection in transformed B cells with established EBV latency and that this suppression is independent from other immune cells expressing TLR-9.

Discussion

Immune activation may be a critical factor in EBV-associated lymphomagenesis. In this work, we examined the effect of immune activation on EBV gene expression. We found that (i) EBV expresses *BZLF-1*, the initiator of lytic EBV infection, and the late lytic genes *gp85* and gp350/220 in CBMC, but not in adult PBMC infected *ex vivo* with EBV, (ii) lower levels of proinflammatory cytokines in CBMC than in adult PBMC are associated with expression of lytic EBV genes and (iii) triggering of TLR-9 suppresses lytic gene expression in CBMC acutely infected *ex vivo* with EBV and in anti-IgG-stimulated chronically infected Akata Burkitt lymphoma cells. Our findings, indeed, identify immune activation as critical factor for the suppression of lytic EBV infection.

We used CBMC to model a state of minimal immune activation compared with PBMC from adults. Importantly, by using primary cells only from EBV-naive individuals, we avoided bias from pre-existing EBV-specific T-cell responses, which may be triggered by *ex vivo* EBV infection. In CBMC, *BZLF-1* and *gp85* mRNA expression and gp350/220 protein expression showed a sharp rise after *ex vivo* EBV infection that persisted over the entire observation time. In adult PBMC, no *BZLF-1*, *gp85* or gp350/220 expression was seen at all, although the fractions of B cells infected with EBV following *ex vivo* infection were similar in CBMC and PBMC. The difference in lytic EBV gene expression cannot be attributed to the higher content of $CD5^+$ cells in CBMC than in PBMC, because $CD5^+$ and $CD5^-$ cells exhibited lytic EBV equally. By contrast, mRNA expression patterns of latent EBV genes were similar in CBMC and PBMC. Extending data published by Hunt *et al.* (1994) the proinflammatory cytokines IL-12, IFN-γ and IL-2 were lower in CBMC than in PBMC before EBV infection. Furthermore, levels of these cytokines in CBMC did not increase to the levels seen in PBMC in response to EBV. Based on these data, we hypothesized that the higher levels of proinflammatory cytokines in PBMC may result in the suppression of *BZLF-1* expression (i.e. that differences in the status of immune activation/maturation are responsible for the profound difference in EBV gene expression between CBMC and PBMC).

The main sources of IL-12 are monocytes and dendritic cells (DCs) (Trinchieri, 2003). As mentioned above, CBMC produce less IL-12 than PBMC (Hunt *et al.*, 1994), and DC derived from neonatal monocytes transcribe much less IL-12 p35 than adult monocytes (Goriely *et al.*, 2001). IFN-γ produced by natural killer (NK) cells (Biron *et al.*, 1999) may, in part, be responsible for the IFN-γ production in CBMC upon EBV encounter *in vitro* (Wilson and Morgan, 2002). The frequency of NK cells in CBMC and PBMC is similar, but NK cells in CBMC have an immature function compared with NK cells in PBMC (Nomura *et al.*, 2001). To determine if the immune activation/maturation deficiencies in IL-12 and IFN-γ production indeed enable *BZLF-1* mRNA expression in CBMC cultures, we added rIL-12 and rIFN-γ to the CBMC

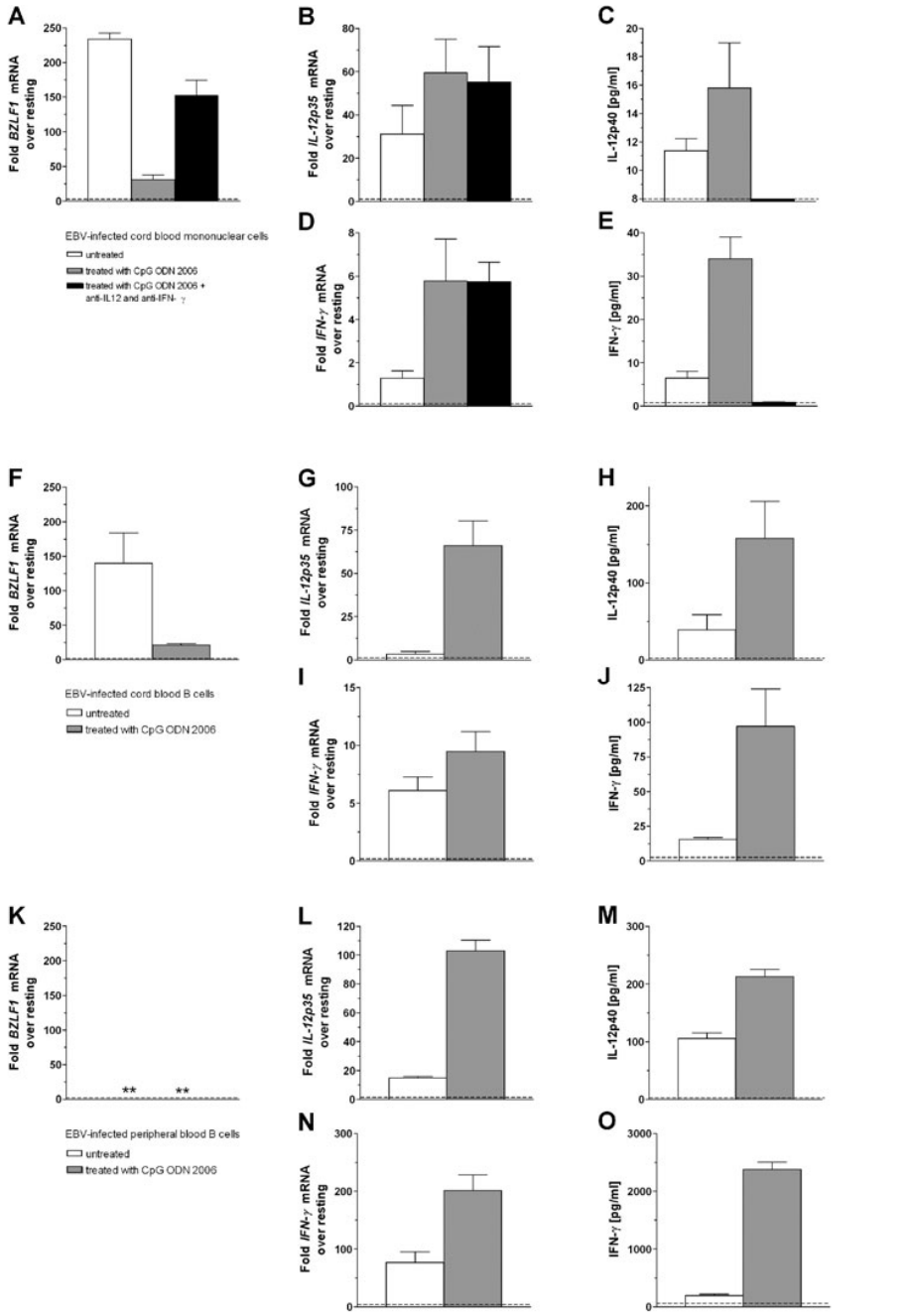

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Fig. 7. *BZLF-1* mRNA expression in CpG ODN 2006-treated CBMC infected *ex vivo* with EBV is dependent on IL-12 and IFN-γ expressed in B lymphocytes.
A. mRNA expression of *BZLF-1* in CBMC infected *ex vivo* with EBV and treated or not with CpG ODN 2006 and with or without anti-IL12 plus anti-IFN-γ blocking antibodies.
B. mRNA expression of *IL-12p35* in CBMC infected *ex vivo* with EBV and treated or not with CpG ODN 2006 and with or without anti-IL12 plus anti-IFN-γ antibodies.
C. IL-12p40 in supernatants of CBMC infected *ex vivo* with EBV and treated or not with CpG ODN 2006 and with or without anti-IL12 plus anti-IFN-γ antibodies.
D. mRNA expression of *IFN-γ* in CBMC infected *ex vivo* with EBV and treated or not with CpG ODN 2006 and with or without anti-IL12 plus anti-IFN-γ antibodies.
E. IFN-γ in supernatants of CBMC infected *ex vivo* with EBV and treated or not with CpG ODN 2006 and with or without anti-IL12 plus anti-IFN-γ antibodies.
F. mRNA expression of *BZLF-1* in $CD19^+$ B cells isolated from cord blood infected *ex vivo* with EBV.
G. mRNA expression of *IL-12p35* in $CD19^+$ B cells isolated from cord blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
H. IL-12p40 in supernatants of $CD19^+$ B cells isolated from cord blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
I. mRNA expression of *IFN-γ* in $CD19^+$ B cells isolated from cord blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
J. IFN-γ in supernatants of $CD19^+$ B cells isolated from cord blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
K. mRNA expression of *BZLF-1* in $CD19^+$ B cells isolated from peripheral blood infected *ex vivo* with EBV.
L. mRNA expression of *IL-12p35* in $CD19^+$ B cells isolated from peripheral blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
M. IL-12p40 in supernatants of $CD19^+$ B cells isolated from peripheral blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
N. mRNA expression of *IFN-γ* in $CD19^+$ B cells isolated from peripheral blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
O. IFN-γ in supernatants of $CD19^+$ B cells isolated from peripheral blood infected *ex vivo* with EBV and treated or not with CpG ODN 2006.
CpG ODN 2006 (1 μM) was added at 0 and 90 h to the EBV-containing culture medium of 2×10^6 CBMC. Anti-IL-12 and anti-IFN-γ antibodies were given to the cultures 1 h before stimulation with CpG ODN 2006. Cells were collected at 96 h. RNA was extracted from two cell pellets per condition, treated with DNase, and reverse-transcribed into cDNA with an oligo-dT15 primer. mRNA expression was measured in duplicate by real-time PCR. Results are means ± SD of fold induction over resting normalized to the housekeeping gene HMBS. The dashed lines indicate the lower limit of detection. One representative experiment of two is shown.

cultures infected *ex vivo* with EBV. Indeed, *BZLF-1* mRNA expression in CBMC decreased significantly, albeit not completely, with rIL-12 and rIFN-γ. The incomplete suppression of *BZLF-1* mRNA expression may be explained by immature cytokine receptor signalling pathways in CBMC (Marodi, 2002) or the need of additional stimuli operative in the innate immune responses (Medzhitov, 2001). Thus, activation of the immune system results in efficient suppression of the initiation of lytic EBV infection.

The anti-inflammatory cytokine TGF-β induces lytic infection in EBV-transformed CBMC-derived cell lines (Liang *et al.*, 2002). Thus, we explored the possibility that

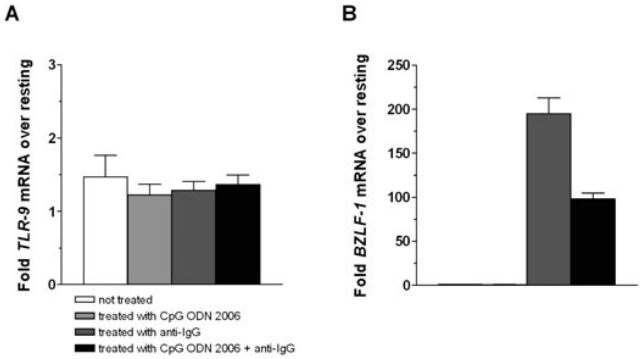

Fig. 8. CpG ODN 2006 suppresses induction of *BZLF-1* mRNA expression in Akata Burkitt lymphoma cells provoked to switch to lytic EBV infection.
A. Expression of *TLR-9* mRNA before and after stimulation with CpG ODN 2006, anti-IgG, or both.
B. Expression of *BZLF-1* mRNA before and after stimulation with CpG ODN 2006, anti-IgG, or both.
Akata cells (1.0×10^6 each) were seeded and treated with or without CpG ODN 2006 (0.5 μM) After 6 h 0.1 μg μl^{-1} polyclonal rabbit anti-human IgG was added to the cultures. Cells were collected at 6 h after anti-IgG treatment. RNA was extracted from one cell pellet per condition, treated with DNase, and reverse-transcribed into cDNA with an oligo-dT15 primer. mRNA expression was measured in duplicate by real-time PCR. Results are means ± SD of fold induction over resting normalized to the housekeeping gene HMBS from three independent experiments.

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the lower levels of IL-12, IFN-γ and IL-2 in CBMC than in PBMC were coupled to higher mRNA levels of *TGF-β*. However, *TGF-β* mRNA expression was lower in CBMC than in PBMC irrespective of *ex vivo* EBV infection, making a contribution of TGF-β to *BZLF-1* expression in CBMC highly unlikely. Another possible reason for the lower IL-12, IFN-γ and IL-2 levels in CBMC than in PBMC could have been increased levels of the anti-inflammatory cytokine IL-10 (Wang *et al.*, 1994). The lower mRNA levels of *IL-10* in CBMC in response to EBV infection compared with in PBMC, however, argued against IL-10 being responsible for the lower levels of proinflammatory cytokines in CBMC. Notably, adding or blocking IL-10 had no effect on lytic EBV gene expression patterns.

We wanted to verify our observation that activation of the immune system results in suppression of *BZLF-1* by activating the innate immune response triggering TLR-9. Indeed, triggering of innate immunity via TLR-9 with CpG ODN 2006 resulted in suppression of *BZLF-1* but not latent EBV gene mRNA expression in acutely *ex vivo* EBV-infected CBMC. Notably, triggering TLR-9 resulted in higher transformation rates of B cells infected *ex vivo* with EBV (Traggiai *et al.*, 2004), but the effect of stimulating TLR-9 of B cells on EBV gene expression was not investigated. Thus, the molecular mechanism(s) resulting in the more efficient transformation rate of B cells when triggering TLR9 may be due to reduction of initiation of lytic EBV infection and thereby reinforce maintenance of EBV latency.

Our results seem to be in conflict with the findings of Liu *et al.* (2005) and Lim *et al.* (2007). Liu *et al.* (2005) reported that truncated thioredoxin (Trx80) inhibits B cell growth in EBV infected CBMC through T cells activated by monocyte derived IL-12. They assessed B cell transformation by EBV by measuring the thymidine incorporation on the 12th day. Patterns of EBV latent and lytic gene expression were not investigated. In contrast, our experiments focusing on acute *ex vivo* EBV infection were limited to 7 days. Through the use of isolated B cells in selected experiments we showed that IL-12 derived from B cells mediated suppression of lytic EBV. We did not assess B cell transformation. Thus, the results of these two studies cannot be directly compared due to the different experimental settings used; they are not mutually contradictory. Future experiments may resolve this enigma. Furthermore, Lim *et al.* (2007), reported that human plasmacytoid DCs regulated immune responses to EBV in humanized NOD-SCID mice resulting in delayed EBV-related mortality. From indirect proof using an inhibitor for triggering TLR-9 they concluded that TLR-9 in part mediated activation of plasmacytoid DCs resulting in anti-EBV-active $CD3^+$ T cells. In this study, PBMC from EBV-seropositive donors were used; thus, the protective effect observed is most likely due to the boosting effect of an adaptive EBV-specific cellular immune response.

Next, we addressed the question whether TLR-9 triggering affects on EBV in chronically infected cells. Chronically EBV-infected cells express latent EBV genes and only very rarely lytic EBV genes. To assess the effects of triggering of TLR-9 on lytic EBV in chronically EBV-infected cells, we used Akata Burkitt lymphoma cells, which undergo lytic EBV infection upon anti-IgG stimulation (Kieff and Rickinson, 2001). Similarly to *ex vivo* acutely infected B cells, TLR-9 triggering suppresses anti-IgG-induced *BZLF-1* expression in Akata cells. This result also indicates that triggering TLR-9 directly affects the EBV gene expression pattern and is not a consequence of indirect effects due to stimulation of other cellular subsets. Of note in this context, *Plasmodium falciparum* malaria pigment hemozoin also stimulates TLR-9 (Coban *et al.*, 2005). Children in areas endemic for both EBV-positive Burkitt lymphoma and malaria are dually infected with EBV and malaria very early in life (Rochford *et al.*, 2005). We show that suppression of lytic EBV via TLRs on and in B cells is specifically linked to triggering of TLR-9 and that suppression of lytic EBV occurs following direct triggering of TLR-9 in B cells. Thus, repeated activation of the innate immunity via TLR-9 (e.g. due to chronic malaria infection) may foster the propagation of latently EBV-infected cells by suppressing lytic EBV infection and thus development of Burkitt lymphoma.

We and others have documented increased plasma EBV DNA levels in patients with IM or EBV-associated lymphoproliferative diseases in immunocompetent and immunodeficient patients (Berger *et al.*, 2001; Ryan *et al.*, 2004) as well as in individuals with malaria (Moormann *et al.*, 2005; Donati *et al.*, 2006). Plasma EBV DNA is sensitive to DNase; this indicates that it is not encapsidated and does not originate from lytic infection but rather from dying latently infected cells (Ryan *et al.*, 2004; Donati *et al.*, 2006). Moorman *et al.* also found elevated EBV DNA blood levels in children with malaria and suggested as likely reasons an increased frequency of latently EBV-infected cells, indirectly due to polyclonal B cell activation or due to suppression of EBV-specific immunity, and that recurrent malaria infections affect either the establishment or maintenance of EBV latency (Moormann *et al.*, 2005). Notably, no *BZLF-1* transcription is found in PBMC from IM patients (Tierney *et al.*, 1994) with high serum levels of IL-12, IFN-γ and IL-2 (Corsi *et al.*, 2004). This is in line with our findings showing IL-12- and IFN-γ-mediated suppression of *BZLF-1* expression. Our findings are further supported by the observations that IFN-γ blocks gammaherpesvirus reactivation from latency (Steed *et al.*, 2006) and nuclear factor κB, activated downstream of TLR-9, inhibits gammaherpesvirus lytic replication (Brown *et al.*, 2003). Thus, we

hypothesize that in states of increased immune activation propagation of EBV is due to promotion of latent rather than of lytic EBV infection. Because control of EBV infection may substantially differ between tissue compartments (Hislop *et al.*, 2005; Donati *et al.*, 2006), in states of immune activation or cellular immune compromise lytic EBV infection may be confined to tissues at mucosal surfaces with excretion of EBV particles.

Experimental procedures

Isolation of mononuclear cells and cell culture

Cord blood mononuclear cells and PBMC from healthy adult EBV-seronegative donors were obtained from heparinized blood by Ficoll-Hypaque (Amersham Biosciences Europe GmbH, Otelfingen, Switzerland) gradient centrifugation. Cells were washed with phosphate-buffered saline (Gibco, Invitrogen Life Sciences, Basel, Switzerland). The EBV-producing cell lines B95.8 (Miller and Lipman, 1973) and B95.8EBfaV-GFP (Speck and Longnecker, 1999), CBMC, PBMC and Akata (Takada, 1984) cells were cultured in RPMI 1640 supplemented with Hepes buffer, L-glutamine, 10% fetal bovine serum, 1 mM sodium pyruvate, 1 mM non-essential amino acids, 100 U ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin sulphate (medium and supplements from Gibco). Informed consent was obtained from subjects or parents before the study. The institutional ethics committee approved the collection and use of clinical material.

Isolation of B cells from CBMC and PBMC

B cells were isolated from CBMC or PBMC by the use of magnetic beads (Miltenyi Biotech, Bergisch-Gladbach, Germany) according to the manufacturer's instructions. Purity of isolated B cells was determined by flow cytometry using anti-human CD19, and anti-human CD3 antibodies for the detection of B cells and eventually remaining T cells. The purity of each separation was above 97%.

Epstein-Barr virus infections ex vivo

After resting overnight, CBMC or PBMC (1×10^7 cells) were infected with supernatants from B95.8 cells or B95.8EBfaV-GFP ($1 \times 10^6\ ml^{-1}$) harvested on day 4 after splitting and filtered using a 0.45 μm sterile filter (Millipore, Cork, Ireland). The cell-free supernatants contained approximately 7 $\log_{10}$ EBV copies ml^{-1}, as evaluated by real-time PCR for EBV DNA (Berger *et al.*, 2001). Infections were performed as described (Tosato, 1991). Briefly, cells were centrifuged, resuspended in 2.5 ml of RPMI and 2.5 ml of B95.8 supernatant, and incubated in 50 ml conical Falcon tubes (BD Biosciences, Basel, Switzerland) at 37°C in a water bath for 2 h. Subsequently, 5 ml of RPMI 1640 were added, and 1 ml aliquots (1×10^6 cells ml^{-1}) were seeded into 24-well plates (BD Biosciences). Cell pellets were centrifuged at 300 *g*, frozen on dry ice, and stored at –80°C.

Assessment of EBV and cytokine gene transcription

RNA extractions were performed with the RNA Easy Extraction kit (Qiagen, Basel, Switzerland), according to the supplier's instructions. RNA was treated with DNase [DNAfree; Ambion (Europe), Huntington, Cambridgeshire, UK] for removal of residual DNA. RNA (1 μg) was reverse transcribed in a total volume of 20 μl with oligo-dT15 primer (Microsynth, Balgach, Switzerland) using Omniscript Reverse Transcription kit (Qiagen). RNase inhibitor (10 units) (RNasin plus, Promega, Catalys AG, Wallisellen, Switzerland) was added to each 20 μl reaction. Real-time PCR (TaqMan) for human *IL-2, IL-12 p35, IFN-*γ*, IL-1*β*, IL-6, IL-8, IL-10, TGF-*β*, TNF-*α genes, EBV nuclear antigen *(EBNA)-1, EBNA-2, latent membrane protein (LMP)-1, LMP-2, BamHI Z fragment (BZLF)*-1, *glycoprotein (gp) 85* (C. Berger, *et al.* submitted), and the housekeeping gene, *hydroxymethylbilane synthase (HMBS)*, were performed according to the supplier's instructions (Applied Biosystems, Foster City, CA, USA) and as described (Bonanomi *et al.*, 2003). The assays were cDNA specific: either the forward or reverse primer or the probe was designed to span exon-exon junctions. Specificity (DNA/cDNA) was tested using RNA before and after DNase treatment and cDNA with or without prior DNase treatment. The assay for human *IL-10* is highly specific and does not detect viral *IL-10* (data not shown). All reactions were performed in duplicate. Each 15 μl reaction contained a mix of the 2× ABI-TaqMan Master Mix (Applied Biosystems), primers (Microsynth) at 300 nM each, the probe (Biosearch Technologies, Novato, CA, USA) at 200 nM, and 1 μl of cDNA template. Ct values obtained for HMBS were used for normalization. Both positive (amplified cDNA sequences of the selected cytokines or EBV genes tested) and negative controls (no template) were included on every plate.

Immunofluorescence

Cord blood mononuclear cells were washed in PBS, transferred to coated slides in a Cytospin 3 centrifuge (Shandon, Histocom, Zug, Switzerland), air dried, fixed with acetone at 4°C, and stored at –20°C. After thawing and before staining, the cells were blocked with 5% goat serum in PBS, incubated with the anti-BZLF-1 antibody (1:40; Clone BZ.1, DakoCytomation, Zug, Switzerland), followed by the secondary goat anti-mouse IgG antibody labelled with the green fluorescent Alexa Fluor 488 dye (Molecular Probes-Invitrogen, Basel, Switzerland). Nuclei were stained with 4,6 diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). Analysis was carried out with the Zeiss AXIOSKOP 2 Mot Plus microscope, the Plan Neofluar 20 ×/0.50 Ph2 objective, the Fluoarc Lamp, the AxioCam MR and the AxioVision 3.1 software (all from Carl Zeiss AG, Oberkochen, Germany). Adobe Photoshop 6.0 was used to magnify the region of interest.

Flowcytometric analyses to determine T-cell activation or fractions of EBV-infected B cells

The cell pellets were resuspended and washed in staining buffer (PBS with 5% FBS and 0.1% sodium azide but without Ca^{+2} or Mg^{+2}). Cells were double stained with an FITC-labelled and a PE-labelled mouse anti-human monoclonal antibody (all from BD Biosciences, if not stated otherwise) at 4°C in the dark for 30 min. As isotype controls, FITC-conjugated anti-mouse IgG_1 (FITC-IgG_1) with PE-conjugated anti-mouse IgG_1 (PE-IgG_1) and FITC-IgG_1 with PE-HLA-ABC were used. Activated T cells were evaluated with FITC-anti-HLA-DR and either PE-anti-CD4 or

PE-anti-CD8. B cells were evaluated with PE-anti-CD19 and Cy5-anti-CD5. Detection of the late lytic EBV glycoprotein gp350/220 was performed using a FITC-labelled anti-EBV gp350/220 antibody (Milan Analytica, La Roche, Switzerland).The samples were analysed using a FACSCalibur (BD Biosciences) equipped with 488 nm and 635 nm lasers for double colour analysis. Events (10 000 per lymphocyte gate) were recorded and analysed with the Cell Quest software (BD Biosciences).

Assessment of cytokine levels

Samples were analysed using multiplex bead analysis that uses microspheres as the solid support for immunoassays (Chen *et al.*, 1999). Cytokine levels were measured according to the manufacturer's instructions (Upstate Biotechnology UK, Buckingham, UK).

Stimulation of CBMC with rIFN-γ, rIL-12, or IL-10 and inhibition of IL-12 or IFN-γ by addition of anti-IL-12, anti-IFN-γ, or IL-10 antibodies

Cord blood mononuclear cells (1×10^6 ml^{-1}) were infected with EBV as described above, but with or without addition of 20 ng ml^{-1} rIL-12, or 10 ng ml^{-1} rIFN-γ, or both (both from R&D Systems, Abingdon, UK), or 1, 10, or 100 pg ml^{-1} IL 10 (Peprotech EC, London, UK). RIL-12, rIFN-γ, or both, or IL-10 were added in 24 h intervals to the cells. CBMC or PBMC (1×10^6 ml^{-1}) were infected with EBV with or without 100 ng anti-IL-12, 1 μg anti-IFN-γ antibodies (both from R&D Systems), or anti-IL-10 antibodies (Biolegend, San Diego, USA).

Stimulation of CBMC, PBMC, or Akata cells with ligands to TLRs

Cells (3 or 5×10^6 cells ml^{-1}) were left uninfected or infected with EBV and were stimulated with TLR ligands added at 0 h and 90 h. Concentrations of TLR ligands were 10 μg ml^{-1} for peptidoglycan (TLR1/2), 20 μg ml^{-1} for lipopolysaccharide (TLR4), 3 μM for R-848 (TLR7/8) and 0.5 μM for CpG ODN 2006 (TLR9) (InvivoGen, San Diego, CA, USA). The cells were kept in culture for a total of 96 h.

Initiation of lytic EBV infection in Akata Burkitt lymphoma cells

Akata cells were split to a concentration of 1×10^6 cells ml^{-1} 24 h before stimulation. Cells (1×10^6 ml^{-1}) were stimulated with 0.1 μg μl^{-1} polyclonal rabbit anti-human IgG (Dako, Zug, Switzerland) and suspended in fresh RPMI 1640. After 6 h, stimulated cells were collected for RNA isolation.

Statistical analyses

The Mann–Whitney *U*-test (two-tailed) was used for comparison of differences between groups. The level of statistical significance was set at $P < 0.05$.

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The TLR profile of plasma cells is distinct from the TLR profile of B-cells and triggering plasma cell TLRs enhances Ig production[1]

Running title: TLR PATTERNS IN B-CELL DEVELOPMENT

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[3] Abbreviations used in this paper: HSC, hematopoietic stem cell; HMBS, hydroxymethylbilan-synthase.

Abstract

Toll-like receptors (TLRs) are key receptors of the innate immune response and show cell-subset specific expression. We hypothesized that expression and function of TLRs are tailored to distinct B-cell development stages. We investigated the mRNA expression of *TLR* genes in hematopoietic stem cells (HSC), in primary B-cells and plasma cells as well as in a panel of B-cell lymphomas arisen from distinct B-cells and B-cell precursors. HSC and plasma cells were unique by their unrestricted expression of *TLR1-TLR9*, but almost absence of *TLR10*. By contrast, mature, i.e. naïve and memory B-cells lacked *TLR3*, *TLR4* and *TLR8* but expressed mRNA of all other *TLR*s. This expression pattern was largely retained in mature B-cell-derived malignancies. Immature B-cell-derived malignancies had no *TLR3* and *TLR4* suggesting that their expression was lost early after HSC commitment to the B-cell lineage. In contrast, TLR8 is lost during transition from immature to mature B-cell. Very importantly, we demonstrate effective functioning of TLRs on plasma cells shown by augmented IgM, IgG, or total Ig production upon TLR ligand stimulation. The property of TLRs to boost Ig production in plasma cells upon pathogen recognition points to a novel role of TLRs which may have therapeutic application such as boosting an antibody response.

Introduction

Toll-like receptors (TLRs) are key recognition structures of the innate immunity (1). They trigger antimicrobial responses subsequent to their ligation by conserved pathogen-associated molecular patterns. The signaling cascade culminates, among others, in the activation of NF-κB which results in transcription of pro-inflammatory genes critical for the innate as well as the adaptive immune responses against pathogens (1). Besides their importance in triggering antimicrobial responses, TLRs play a role in autophagy (2), hematopoiesis (3) and neutrophil activation (4). For each of the ten known human TLRs (TLR1-TLR10) at least one distinct ligand derived from microbial pathogens has been identified except for TLR10.

Cell subpopulations exhibit specific TLR expression patterns (5, 6) indicating that TLR expression is tailored to distinct cellular tasks and functions. Furthermore, TLR expression pattern depends on the developmental stage as exemplified by the developmental-dependent degree of *TLR1-TLR5* expression in dendritic cells (7) and *TLR9* in B-cells (8). Delineation of TLR expression during human B-cell development is so far incomplete (9). An analysis of all known TLRs of human B-cells at their different maturation stages with particular emphasis on plasma cells will give a comprehensive image of the TLR profile which is the basis for functional studies.

In the current study, we hypothesized that expression and function of TLRs are tailored to distinct stages of B-cell development. Therefore, we investigated expression of *TLR1-TLR10* at distinct B-cell developmental stages including hematopoietic stem cells (HSC),[3] naïve and memory B-cells as well as plasma cells. The impact of plasma cell TLR triggering on Ig production has not been investigated. Thus, we addressed also this functional issue.

Materials and Methods

Cells and subpopulations

Cells were isolated from cord blood, peripheral blood or tonsils as described (10-12). The study was approved by the local ethics committee and written informed consent was obtained for all tissue obtained. Cord blood HSC, B-cells and plasma cells were isolated using CD34 microbeads, the B-cell isolation kit II and CD138 microbeads, respectively according to the instructions of the manufacturer (Miltenyi Biotech, Bergisch Gladbach, Germany). Further separation of B-cells into naive and memory B cells was performed using the naive-B-cell isolation kit (Miltenyi Biotech) or CD27 microbeads (Miltenyi Biotech) (12) . Isolated cell populations used for experiments were always >95% pure as determined by flow cytometry. Leukemia cell RNA was obtained from the repository of the Division of Oncology, Children's Hospital of Zurich (13). Frozen lymphoma tissue was generously provided from the Institute of Surgical Pathology of Zurich (approved by the local ethics committee). The leukemias and lymphomas were characterized according to the WHO classification 2001 (14).

Quantitative real-time PCR

Quantitative real-time PCR was performed for *TLR9* and the housekeeping gene *hydroxymethylbilane-synthase (HMBS)* as described (10, 12). *TLR10* was analyzed using primer/probe on demand (Hs01935337_s1, Assay-on-demand gene expression product, Applied Biosystems, Foster City, CA). SYBR Green primers for *HMBS* and *TLR1-TLR8* were described earlier (15).

Flow cytometry

Flow cytometry using fluorochrome-conjugated monoclonal antibodies to human CD34, CD19, CD27, CD138, IgM or IgG (BD Biosciences, Basel, Switzerland) was executed on a Cytomics FC500 instrument (Beckman Coulter, Nyon, Switzerland); data were analyzed with FlowJo software (Treestar, Ashland, OR).

Intracellular Ig staining and ELISA

Tonsillar plasma cells were either left untreated or stimulated with 10 μg/mL peptidoglycan (TLR1/2 ligand; Fluka, Buchs, Switzerland), 1 μg/mL poly(I:C) (TLR3 ligand; InvivoGen, San Diego, California), 10 ng/mL LPS (TLR4 ligand; Sigma-Aldrich, Buchs, Switzerland), 10 ng/mL Flagellin (TLR5 ligand), 3 μM R-848 (TLR7/8 ligand; InvivoGen), or 2 μM CpG ODN 2006 (TLR9 ligand; Eurogentec, Köln, Germany) respectively. 72 hours after stimulation, cells were harvested, fixed, permeabilized and stained. Intracellular staining of IgM and IgG on plasma cells was performed using fluorchrom-conjugated monoclonal antibodies and the BD Cytofix/Cytoperm kit (both from BD Biosciences) according to the manufacturer's instructions.

The total amount of secreted Ig was determined by an in-house ELISA: briefly, 96-well microtiter plates were coated with 10 μg/mL Protein G (Calbiochem, Dietlikon, Switzerland) and kept overnight at room temperature in a humid chamber. The Protein G was diluted in a carbonate–bicarbonate buffer, pH 9.6. The plates were washed four times with PBS and incubated with 200 μL 3% BSA in PBS per well for 1 h at room temperature. After discarding the blocking buffer, 50 μL supernatant of the plasma cell samples or serial dilutions of human Ig (NIBSC, Hertfordshire, UK) as reference were added to each well and allowed to react for 30 min at 37 °C. After three washing steps, peroxidase-labeled sheep anti-human Ig (Millipore, Munich, Germany) was incubated for 30 min at 37 °C. After three washing steps, 100 μL of 3,3′,5,5′-tetramethyl-benzidine substrate (Mabtech, Hamburg, Germany) were

added and incubated for 30 min at 37 °C in the dark. Reactions were stopped by the addition of 50 µL 1 M citrate. The optical density was determined photometrically at 450 nm with 620 nm as reference filter.

Results

Pattern of TLR mRNA expression changes during B-cell development

The aim of this work was to investigate the expression pattern of *TLR1-TLR10* at distinct B cell developmental stages. Thus, we analyzed *TLR* expression in HSC, in naïve and memory B cells as well as in plasma cells from the palatine tonsils. In the absence of reliable antibodies to quantify TLR at the protein level, we used quantitative real-time PCR. We found that HSC and tonsillar plasma cells expressed well all *TLR*s with the exception of *TLR10* (Fig. 1). In contrast to HSC and plasma cells, we found a complete absence of *TLR*3, *TLR4* and *TLR8* expression in naïve and memory B-cells while the other *TLR*s were expressed to a variable degree (Fig. 1). Next, we asked whether circulating plasma cells differ in their *TLR* expression from their tonsillar counterparts to exclude that plasma cells exhibit distinct *TLR* expression patterns after exiting secondary lymphoid organs. We found that plasma cells from the peripheral blood exhibited the same expression pattern as tonsillar plasma cells (Fig. 2).

Lymphoid malignancies derived from peripheral B-cells largely retain TLR expression patterns from the developmental stage of origin

B-cells at each developmental stage may give rise to malignant B-cells, and malignant B-cells often retain characteristics of the developmental stage from which they derive (16). Thus, we were particularly interested whether *TLR* expression may be one of these characteristics. To verify this we analyzed 19 malignancies derived from 4 distinct mature, i.e. peripheral B-cell developmental stages which would allow comparison to the results reported above for normal primary cells. All these malignancies showed almost a complete absence of *TLR*3, *TLR4* and

TLR8 expression while the other *TLR*s were expressed. The only exception to this pattern was follicular lymphoma lacking *TLR1*. By contrast, plasma cell-derived multiple myeloma exhibited no *TLR10* but all other *TLRs* (Fig. 3). Thus, *TLR* mRNA expression patterns in B-cell malignancies derived from mature, i.e. peripheral B-cell developmental stages were similar to those in normal cellular counterparts. This indicates that developmental stage related TLR expression is largely retained during malignant transformation.

Bone marrow-derived lymphoid malignancies exhibit TLR mRNA expression patterns distinct from hematopoietic stem cells

In a next step, we wanted to identify the *TLR* profile in distinct primary B-cell developmental stages from the bone marrow. However, for reasons of good clinical practice as well as for technical reasons, it is very difficult to obtain these cell subsets in adequate numbers from humans. Since there was a very good accordance of *TLR* profiles between primary mature B-cells and B-cell lymphomas derived from mature B-cells, we assumed that *TLR* expression will also be retained during malignant transformation of primary B-cells from the bone marrow, i.e. immature B-cells, and thus assessing the *TLR* profile of those malignancies would complement our analysis.

Of 21 bone marrow-derived B-cell malignancies, none expressed *TLR3* or *TLR4* mRNA but expressed all other *TLR*s (Fig. 3). *TLR5* mRNA was present in very low amounts. The only exception was common acute lymphoblastic leukemia which expressed minimal amounts of *TLR4* and no *TLR8* mRNA. Hence, bone marrow-derived B-cell malignancies exhibited *TLR* expression patterns similar to peripheral B-cells and not to HSC. The lack of expression of *TLR3* and *TLR4* in bone marrow-derived B-cell malignancies speaks in favor that loss of *TLR3* and *TLR4* expression occurs very early after HSC commit to B-cell lineage. In contrast,

the presence of *TLR8* in B-cell precursors and lack of *TLR8* in mature cells suggest that its loss occurs when immature B-cells develop to mature B-cells.

Engagement of TLRs on plasma cells increases Ig production and secretion

Based on the TLR profile of plasma cells, we wondered whether triggering TLR affects the main function of these cells, i.e. Ig production and secretion. Indeed, purified plasma cells resulted in increased production of intracellular IgM (Fig. 4A, C) and IgG (Fig. 4B, D) as well as secretion of total Ig (Fig. 4E) in response to ligands binding TLR1/2 and TLR3 or TLR9 and TLR1/2, TLR3, or TLR5, respectively. This increased Ig production/secretion suggests that pattern recognition receptors of plasma cells may be critical in regulating the strength of an adaptive antibody-mediated immune response and that pattern recognition receptors have additional function beyond of their role in the innate immune response. The omnipresent *TLR1-TLR9* mRNA expression contrasts the selective strong responses to TLR1/2, TLR3, TLR5, or TLR9 speaking in favor of a partial uncoupling of TLR expression from its function. Here, we provide the first evidence that engagement of TLRs on plasma cells increases immunoglobulin production (Fig. 4).

Discussion

In the present study, we aimed to assess the TLR expression patterns at distinct human B-cell developmental stages including HSC, naïve and memory B-cells as well as plasma cells and to analyze the impact of plasma cell TLR engagement. Indeed, we demonstrated that TLR expression patterns display notable changes during peripheral B-cell development, plasma cells exhibit TLR expression distinct from naïve and memory B-cells but similar to HSC, and engagement of plasma cell TLRs results in augmented Ig production and secretion by these cells. These results suggest that TLR expression is tailored to cellular developmental stage function.

In agreement with published data (5, 6, 17), we found a complete absence of *TLR*3, *TLR4* and *TLR8* expression in naïve and memory B-cells while the other *TLR*s were expressed in those cells in various amounts. We complemented the characterization of the TLR profile in the B-cell lineage by examining plasma cells: we found that plasma cells express all TLRs with the exception of TLR10 which is very weakly expressed. In contrast, naïve and memory B-cells show a high expression of *TLR10*. Strikingly, HSC do also virtually not express *TLR10*. Notably, lack of acceptable specificity of antibodies to TLRs (not shown) precluded analysis at the protein level. Triggering TLR resulted in increased production of IgM and IgG as well as secretion of soluble Ig by plasma cells which also speaks in favor of the functional integrity of TLRs and their immunological significance. This increased Ig production/secretion points to a novel role of TLRs: TLRs are known for their role in the innate immune responses as well as for linking the innate and adaptive immune responses by enhancing cellular activation, upregulation of the MHC class molecules and release of cytokines; the fact that TLR agonists directly engage in the generation and strength of antibody production uncovers a novel role which may have therapeutic application, e.g., boosting an antibody response. Noteworthy, we observed preferential production of IgM

following triggering of TLR1/2 and of IgG following triggering of TLR3 or TLR9, respectively, as well as a preferential secretion of Ig following triggering of TLR1/2, TLR3, or TLR5. This may be either due to preferential recognition and specialized function of TLRs in plasma cells in response to distinct conserved motifs or due to the preferential isotype Ig responses that the plasma cells we purified had. Previous analyses of TLR function in B-cell lineages have shown that naïve and memory B-cells respond to TLR agonists by sustaining or increasing their TLR expression or inducing expression of proinflammatory cytokines such as IL-8 (5, 6, 17). Thus, the data we report outline a higher complexity of TLR function in B-cell immunology.

Our observation on TLR expression by plasma cells from tonsils or peripheral blood is divergent from the one reported for plasma cells from the bone marrow which show largely absence of TLR expression (18). It is generally agreed that the bone marrow is the primary site of long-lived plasma cells which are responsible for at least some persistent antibodies, independent of memory B cells and antigen (19). Thus, given their continuous antibody production, long-lived plasma cells in the bone marrow may not need a boosting via TLR triggering and therefore TLR expression by bone marrow plasma cells may be dispensable.

The cell-subset specific TLR expression observed here suggests that HSC and periphery plasma cells need the expression of *TLR1*-*TLR9* whereas B-cells from secondary lymphoid organs need expression of *TLR1*, *TLR2*, *TLR5*-*TLR7*, *TLR9* and *TLR10*. We speculate that the distinct TLR profile of HSC is critical for their differentiation towards a specific cell subset depending upon the encountered pathogen recognition pattern to optimize pathogen-driven immune responses. Indeed, recent work in mice (3) and humans (20) has demonstrated that TLR signals bias HSC toward myelopoiesis directly by replacing endogenous cytokines normally required for the survival, proliferation, and development of hematopoietic progenitors. In naïve and memory B-cells TLR triggering seems to be restricted to sustaining

or increasing their TLR expression or inducing expression of pro-inflammatory cytokines such as IL-8 (5, 6, 17). Finally, as we show, peripheral plasma cell TLR engagement augments Ig release. Nevertheless, other so far not identified TLR functions in these cell subsets may exist.

It is extremely difficult to obtain distinct bone marrow-derived, i.e. immature B-cell developmental stages from healthy donors. We have validated that mature (periphery) B-cells largely retain TLR expression upon malignant transformation. We found expression of *TLR1, TLR2, TLR5-7, TLR9,* and *TLR10* in the vast majority and in the three distinct developmental stages of origin in mature (peripheral) B-cell-derived malignancies. This comprehensively expands on the reported *TLR7* and *TLR9* expression by B-cell chronic lymphocytic leukemia (21, 22) and *TLR9* by non-Hodgkin lymphoma (23). By contrast, plasma cell-derived multiple myeloma exhibited no *TLR10* but all other *TLRs*, expanding on the reported expression of *TLR4*, *TLR7*, and *TLR9* in multiple myeloma (24). These observations may justify the assumption that malignant B-cells derived from immature (bone marrow) B-cells may have retained TLR expression of their origin cells. We took advantage of the possibility to assess TLR expression in primary lymphoid tumor cells derived from different bone marrow B-cell developmental stages. Virtually none of these malignancies expressed *TLR3* or *TLR4* mRNA but all expressed all other *TLRs.* Thus, bone marrow-derived B-cell malignancies exhibited *TLR* expression patterns similar to peripheral naïve and memory B-cells except for *TLR8* and distinct from HSC. This may suggest that loss of *TLR3* and *TLR4* expression occurs very early after HSC commit to B-cell lineage and loss of *TLR8* expression during the transition from immature to mature B cell. Notably, engagement of TLRs on B-cell tumors has been shown to induce diverse responses including apoptosis, growth and survival signals, or increased immunogenicity (21-24). Thus, it will be challenging to individually characterize tumors prior to application of treatments using TLR triggering.

In conclusion, we report that TLR expression differ according to B-cell developmental stage. TLR1-TLR9 may promote augmentation of antibody production by plasma cells which may have therapeutic application. By contrast, TLR10 for which no ligand has been recognized so far and which is virtually not expressed in HSC or plasma cells may functionally be linked to specific naïve and memory B-cell actions rather than simple pathogen sensing. Finally, TLR expression is largely retained during malignant B-cell transformation, and this may impact on tumor behavior, but may also be exploited for new treatment strategies.

It is tempting to speculate that TLR10 mediates a unique function, e. g. interaction with self proteins and thereby homing of naïve and memory B-cells to secondary lymphoid organs. This speculation is based on TLR10 being expressed at the cell surface (1) and not by cell lineages other than B-cell (5, 6, 9, 17), B-cell differentiation to plasma cell requiring among others changes in cell-surface proteins and homing (19), on findings of TLR4 recognizing host structures (25) and of TOLL guiding dorsal-ventral cell orientation in *Drosophila* (26).

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Disclosures

The authors have no financial conflict of interest.

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Figure Legends

FIGURE 1. mRNA expression levels of *TLR1-TLR10* show distinct regulation patterns during B-cell development. mRNA expression profiling of *TLR1-TLR10* in hematopoietic stem cells (HSC) isolated from cord blood and naïve and memory B-cells and terminally differentiated plasma cells from tonsils. HSC and plasma cells were isolated by positive selection using CD34- and CD138-microbeads, respectively. Naïve B-cells and memory B-cells were isolated using the naïve B-cell isolation kit and a combination of B-cell isolation kit II and CD27 microbeads, respectively. Expression of *TLR1-TLR10* and housekeeping gene *hydroxymethylbilane-synthase (HMBS)* mRNA was monitored by quantitative real-time PCR. Results shown are means ± SD of three biological replicates of one out of three representative experiments. * denotes not detectable.

FIGURE 2. Plasma cells from peripheral blood express mRNA of *TLR1-TLR9* but only very little of *TLR10* similar to plasma cells from palatine tonsils. Plasma cells were isolated from peripheral blood or palatine tonsils by positive selection using CD138-microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany). Following RNA extraction, DNAse treatment, and reverse transcription as reported, expression of *TLR* and housekeeping gene *hydroxymethylbilane-synthase (HMBS)* mRNA was monitored by quantitative real-time PCR. Results shown are means ± SD of three biological replicates of one out of three representative experiments.

FIGURE 3. Periphery-derived B-cell malignancies largely retain *TLR*s mRNA expression patterns observed in normal B-cells from similar developmental stages. mRNA expression profiling of *TLR1-TLR10* in various bone-marrow and periphery B-cell developmental stages-derived human B-cell malignancies. cALL: common acute lymphoblastic leukemia (n = 11);

pre-B ALL (n = 4); pro-B ALL (n = 2); B-ALL (n = 3); B-CLL/SLL u: B-cell chronic lymphocytic leukemia / small lymphocytic leukemia unmutated (n = 1); MZL: marginal zone lymphoma (n = 6); B-CLL/SLL m: B-cell chronic lymphocytic leukemia / small lymphocytic leukemia mutated (n = 1); FL: follicular lymphoma (n = 5); DLBCL (GC): diffuse large B cell lymphoma germinal center type (n = 3); BL: Burkitt's lymphoma (n = 1); DLBCL (AC): diffuse large B cell lymphoma activated cell type (n = 2); MM: plasma cell myeloma (n = 1). Expression of *TLR1-TLR10* and housekeeping gene *hydroxymethylbilane-synthase (HMBS)* mRNA was monitored by quantitative real-time PCR. Results are mean ± SD of 3-11 independent primary tumor biopsy samples. Dashed vertical lines separate different B cell developmental stages. * denotes not detectable.

FIGURE 4. Triggering of TLRs on plasma cells induces increased production of Ig. Intracellular (A) IgM and (B) IgG expression and mean fluorescence intensity of (C) IgM and (D) IgG in, and (E) secretion of Ig by plasma cells isolated from tonsils. Results shown are means ± SD of three biological replicates of one out of three representative experiments. * $P < .001$; ** $P < .01$; *** $P < .05$, by Kruskall-Wallis and Dunn's multicomparison test.

Figure 1

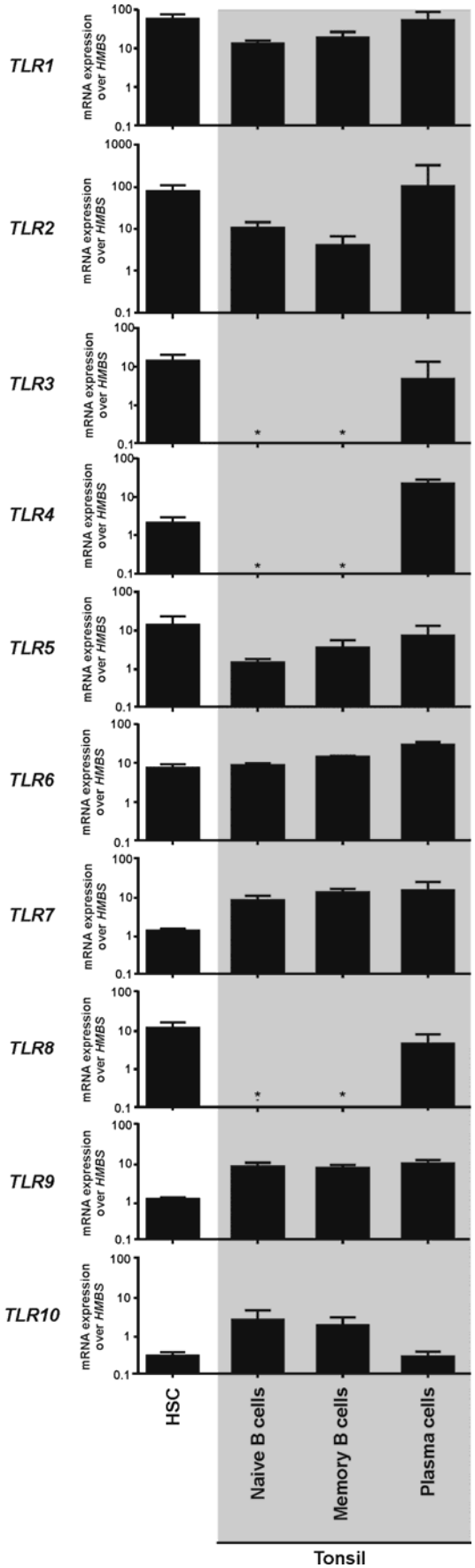

TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR7
TLR8
TLR9
TLR10
mRNA expression over HMBS
100
10
1
0.1
1000
*
HSC
Naive B cells
Memory B cells
Plasma cells
Tonsil

Figure 2

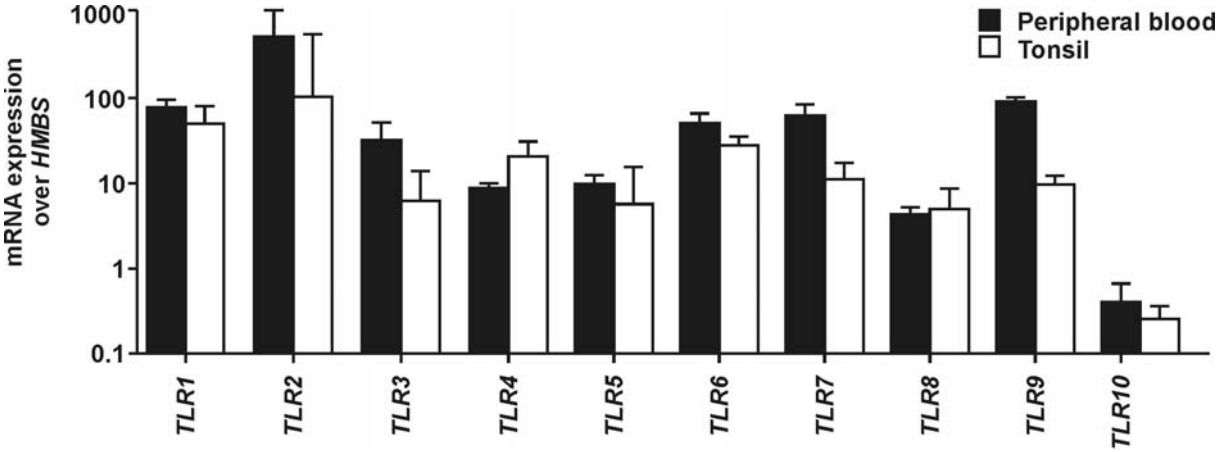

Peripheral blood
Tonsil
mRNA expression over HMBS
1000
100
10
1
0.1
TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR7
TLR8
TLR9
TLR10

Figure 3

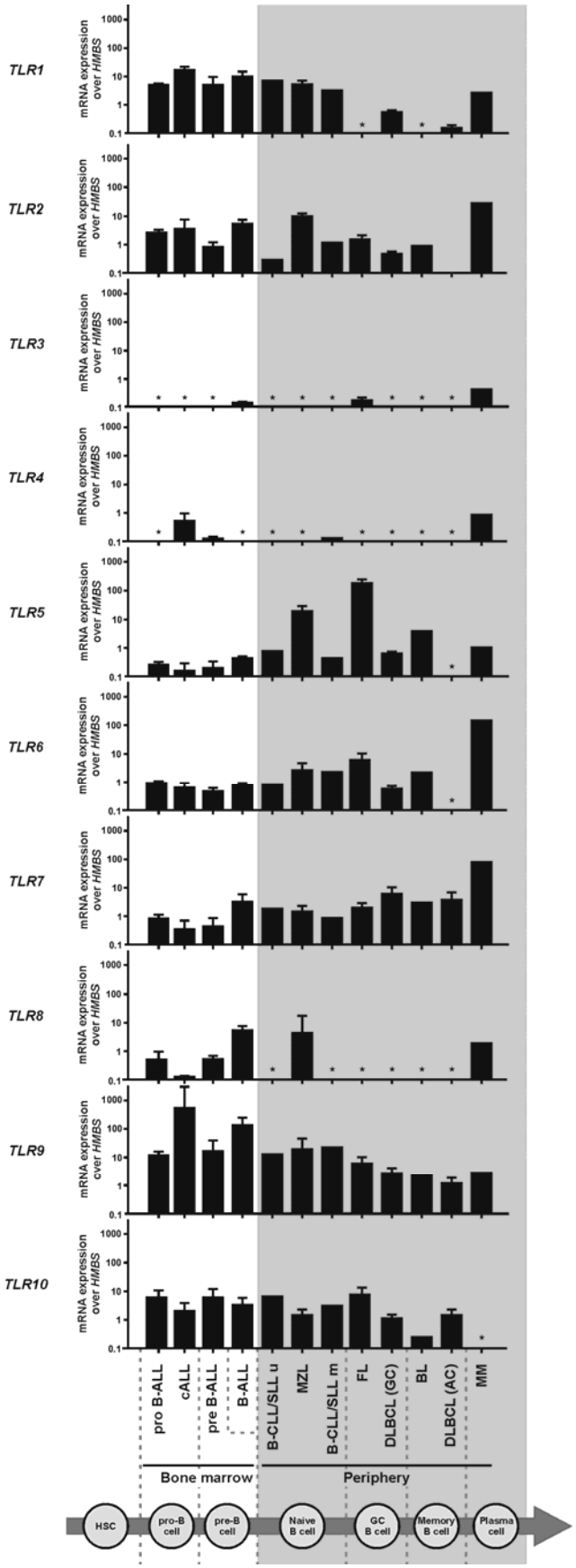
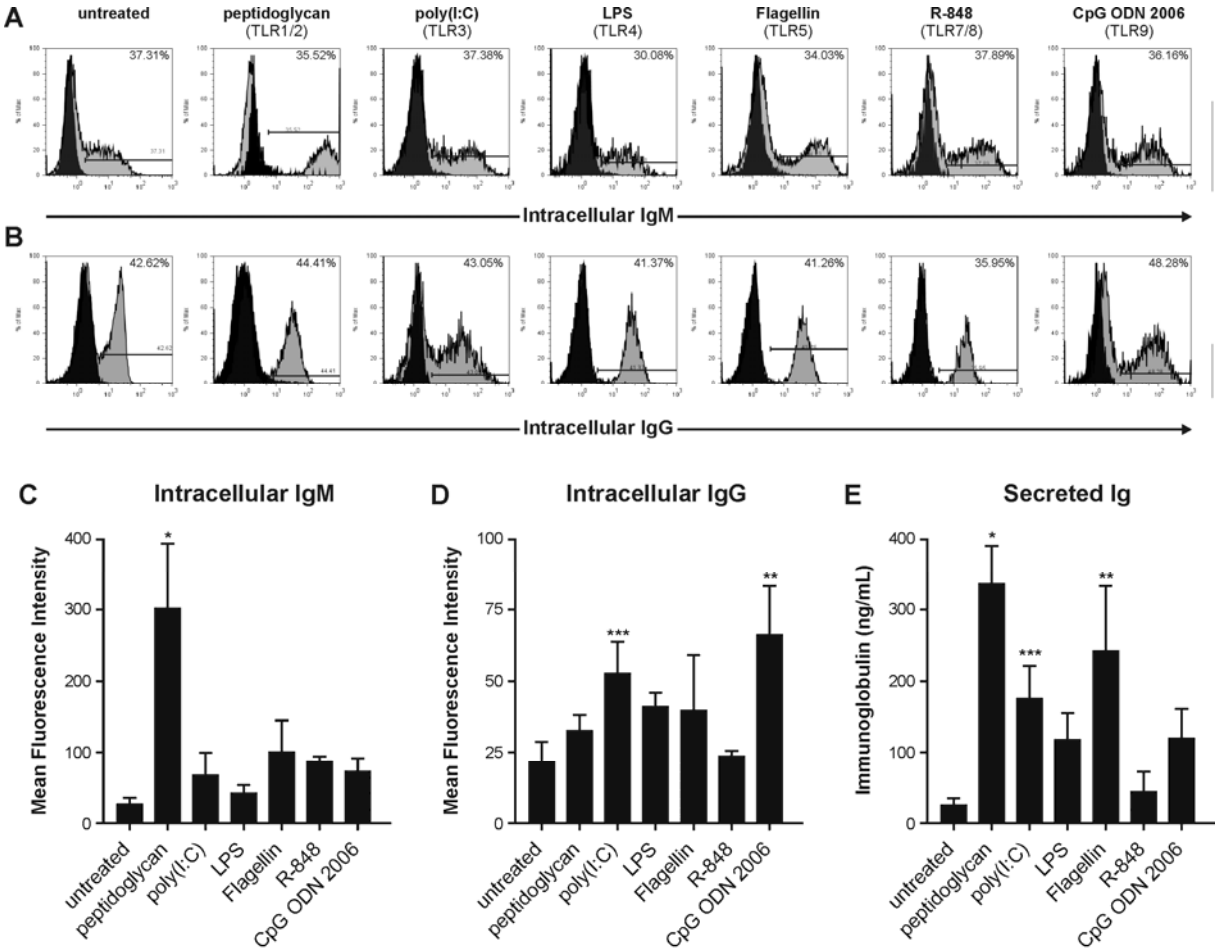

TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR7
TLR8
TLR9
TLR10
mRNA expression over HMBS
1000
100
10
1
0.1
pro B-ALL
cALL
pre B-ALL
B-ALL
B-CLL/SLL u
MZL
B-CLL/SLL m
FL
DLBCL (GC)
BL
DLBCL (AC)
MM
Bone marrow
Periphery
HSC
pro-B cell
pre-B cell
Naive B cell
GC B cell
Memory B cell
Plasma cell

Figure 4


A
untreated
peptidoglycan (TLR1/2)
poly(I:C) (TLR3)
LPS (TLR4)
Flagellin (TLR5)
R-848 (TLR7/8)
CpG ODN 2006 (TLR9)
37.31%
35.52%
37.38%
30.08%
34.03%
37.89%
36.16%
Intracellular IgM
B
42.62%
44.41%
43.05%
41.37%
41.26%
35.95%
48.28%
Intracellular IgG
C
Intracellular IgM
Mean Fluorescence Intensity
D
Intracellular IgG
Mean Fluorescence Intensity
E
Secreted Ig
Immunoglobulin (ng/mL)
untreated
peptidoglycan
poly(I:C)
LPS
Flagellin
R-848
CpG ODN 2006

9. Microarray Gene Lists

9.1. Genes regulated exclusively in naïve B cells upon EBV transformation

GENES UPREGULATED EXCLUSIVELY IN NAIVE B CELLS UPON TRANSFORMATION BY EBV

Probe Set ID	Fold Change	Gene Symbol	Gene Title	Pathway	go biological process term	go molecular function term
227080_at	98.27	ZNF697	zinc finger protein 697	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
215076_s_at	88.55	COL3A1	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	Inflammatory_Response_Pathway	phosphate transport /// cell-matrix adhesion /// transforming growth factor beta receptor signaling pathway /// integrin-mediated signaling pathway /// heart development /// response to radiation /// peptide cross-linking /// platelet activation /// collagen fibril organization /// collagen fibril organization /// collagen biosynthetic process /// collagen biosynthetic process /// response to cytokine stimulus /// wound healing /// wound healing /// skin development /// negative regulation of immune response	integrin binding /// integrin binding /// structural molecule activity /// extracellular matrix structural constituent /// extracellular matrix structural constituent /// protein binding
202765_s_at	76.77	FBN1	fibrillin 1	---	skeletal development /// heart development /// blood coagulation	transmembrane receptor activity /// extracellular matrix structural constituent /// extracellular matrix structural constituent /// extracellular matrix structural constituent /// binding /// calcium ion binding /// calcium ion binding /// calcium ion binding /// protein binding
207315_at	36.92	CD226	CD226 molecule	---	cytokine production /// cell adhesion /// cell adhesion /// signal transduction	receptor activity /// protein binding
226497_s_at	30.78	---	CDNA FLJ35153 fis, clone PLACE6010765	---	---	---
241898_at	30.77	---	Transcribed locus	---	---	---
221524_s_at	30.32	RRAGD	Ras-related GTP binding D	---	---	nucleotide binding /// protein binding /// GTP binding
232098_at	28.47	DST	dystonin	---	cytoskeleton organization and biogenesis /// cell cycle arrest /// cell adhesion /// integrin-mediated signaling pathway /// actin cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis	actin binding /// integrin binding /// structural constituent of cytoskeleton /// structural constituent of cytoskeleton /// calcium ion binding /// protein binding /// protein binding /// protein C-terminus binding /// actin filament binding
211828_s_at	26.6	TNIK	TRAF2 and NCK interacting kinase	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// response to stress /// protein kinase cascade /// JNK cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// sma GTPase regulator activity /// ATP binding /// ATP binding /// kinase activity /// transferase activity
214978_s_at	25.94	PPFIA4	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4	---	cell communication	receptor activity /// protein binding
230425_at	24.61	EPHB1	EPH receptor B1	---	protein amino acid phosphorylation /// signal transduction /// transmembrane receptor protein tyrosine kinase signaling pathway /// nervous system development	nucleotide binding /// protein kinase activity /// protein tyrosine kinase activity /// transmembrane receptor protein tyrosine kinase activity /// transmembrane receptor protein tyrosine kinase activity /// receptor activity /// ephrin receptor activity /// protein binding /// ATP binding /// kinase activity /// transferase activity
203139_at	24.44	DAPK1	death-associated protein kinase 1	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// apoptosis /// anti-apoptosis /// induction of apoptosis /// signal transduction /// protein kinase cascade /// protein kinase cascade /// induction of apoptosis by extracellula signals /// positive regulation of apoptosis	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein binding /// protein binding /// calmodulin binding /// calmodulin binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity
209933_s_at	24.15	CD300A	CD300a molecule	---	immune response /// cell adhesion	receptor activity /// protein binding
212089_at	21.37	LMNA	lamin A/C	---	---	structural molecule activity /// protein binding
210519_s_at	20.29	NQO1	NAD(P)H dehydrogenase, quinone 1	---	xenobiotic metabolic process /// nitric oxide biosynthetic proces /// synaptic transmission, cholinergic /// response to toxin	NAD(P)H dehydrogenase (quinone) activity /// NAD(P)H dehydrogenase (quinone) activity /// cytochrome-b5 reductase activity /// electron carrier activity /// oxidoreductase activity /// coenzyme binding
212489_at	20.11	COL5A1	collagen, type V, alpha 1	---	phosphate transport /// cell adhesion	structural molecule activity /// extracellular matrix structural constituent /// heparin binding
210664_s_at	20.04	TFPI	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	---	blood coagulation /// blood coagulation	endopeptidase inhibitor activity /// endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity /// protease inhibitor activity
204083_s_at	19.11	TPM2	tropomyosin 2 (beta)	Striated_muscle_contraction	regulation of ATPase activity	actin binding /// actin binding /// structural constituent of muscle
228496_s_at	17.7	CRIM1	Cysteine rich transmembrane BMP regulator 1 (chordin-like)	---	regulation of cell growth /// nervous system development	enzyme inhibitor activity /// serine-type endopeptidase inhibitor activity /// insulin-like growth factor receptor activity /// insulin-lik growth factor binding
214239_x_at	17.38	PCGF2	polycomb group ring finger 2	---	transcription /// regulation of transcription, DNA-dependent	DNA binding /// transcription factor activity /// protein binding /// zinc ion binding /// metal ion binding
209946_at	17.22	VEGFC	vascular endothelial growth factor C	---	angiogenesis /// positive regulation of neuroblast proliferation /// substrate-bound cell migration /// signal transduction /// multicellular organismal development /// cell proliferation /// positive regulation of cell proliferation /// positive regulation of cell proliferation /// organ morphogenesis /// morphogenesis of embryonic epithelium /// cell differentiation /// vascular endothelial growth factor receptor signaling pathway	growth factor activity /// vascular endothelial growth factor receptor 3 binding
219232_s_at	17.1	EGLN3	egl nine homolog 3 (C. elegans)	---	apoptosis /// protein metabolic process	iron ion binding /// protein binding /// oxidoreductase activity /// oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen /// oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors /// L-ascorbic acid binding /// metal ion binding
1552390_a_at	16.95	C8orf47	chromosome 8 open reading frame 47	---	---	---
225116_at	16.7	HIPK2	homeodomain interacting protein kinase 2	---	transcription /// regulation of transcription, DNA-dependent /// protein amino acid phosphorylation /// apoptosis /// induction of apoptosis by intracellular signals /// virus-host interaction /// positive regulation of transforming growth factor beta receptor signaling pathway /// positive regulation of JNK cascade	nucleotide binding /// transcription corepressor activity /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// protein binding /// ATP bindin /// kinase activity /// transferase activity /// virion binding
212254_s_at	16.56	DST	dystonin	---	cytoskeleton organization and biogenesis /// cell cycle arrest /// cell adhesion /// integrin-mediated signaling pathway /// actin cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis	actin binding /// integrin binding /// structural molecule activity /// structural constituent of cytoskeleton /// structural constituent of cytoskeleton /// calcium ion binding /// protein binding /// protein binding /// protein C-terminus binding /// actin filament binding
206117_at	16.32	TPM1	tropomyosin 1 (alpha)	Striated_muscle_contraction	cell motility /// regulation of muscle contraction /// regulation of heart contraction	actin binding /// structural constituent of cytoskeleton /// structural constituent of muscle
1555060_a_at	15.77	IKZF2	IKAROS family zinc finger 2 (Helios)	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
231875_at	15.56	KIF21A	kinesin family member 21A	---	microtubule-based movement	nucleotide binding /// motor activity /// microtubule motor activity /// ATP binding
200951_s_at	14.74	CCND2	cyclin D2	Cell_cycle_KEGG /// G1_to_S_cell_cycle_Reactome /// Ovarian_Infertility_Genesis /// Wnt_signaling	positive regulation of protein amino acid phosphorylation /// cell cycle /// positive regulation of cell proliferation /// positive regulation of cyclin-dependent protein kinase activity /// cell division	protein binding /// protein binding /// protein kinase binding /// protein kinase binding
202746_at	14.41	ITM2A	integral membrane protein 2A	---	---	---
1566194_at	14.36	---	Transcribed locus	---	---	---
201403_s_at	14.34	MGST3	microsomal glutathione S-transferase 3	---	lipid metabolic process /// signal transduction	glutathione transferase activity /// glutathione transferase activit /// peroxidase activity /// transferase activity
209526_s_at	14.3	HDGFRP3	hepatoma-derived growth factor, related protein 3	---	cell proliferation	growth factor activity
201243_s_at	14.19	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	Calcium_regulation_in_cardiac_cells	response to hypoxia /// transport /// transport /// ion transport /// potassium ion transport /// sodium ion transport	sodium:potassium-exchanging ATPase activity /// sodium:potassium-exchanging ATPase activity /// potassium ion binding /// sodium ion binding
227556_at	14.18	NME7	non-metastatic cells 7, protein expressed in (nucleoside-diphosphate kinase)	Calcium_regulation_in_cardiac_cells	GTP biosynthetic process /// UTP biosynthetic process /// CTP biosynthetic process /// nucleotide metabolic process	nucleotide binding /// magnesium ion binding /// nucleoside diphosphate kinase activity /// ATP binding /// kinase activity /// transferase activity /// metal ion binding

203215_s_at	14.08	MYO6	myosin VI	---	transport /// intracellular protein transport /// endocytosis /// endocytosis /// endocytosis /// sensory perception of sound /// sensory perception of sound /// sensory perception of sound /// protein transport /// actin filament-based movement /// actin filament-based movement /// DNA damage response, signal transduction by p53 class mediator /// positive regulation of transcription from RNA polymerase II promoter /// regulation of secretion	nucleotide binding /// motor activity /// motor activity /// actin binding /// actin binding /// protein binding /// calmodulin binding /// calmodulin binding /// calmodulin binding /// ATP binding /// protein homodimerization activity /// ADP binding /// actin filament binding /// actin filament binding /// minus-end directed microfilament motor activity
213107_at	13.61	TNIK	TRAF2 and NCK interacting kinase	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// response to stress /// protein kinase cascade /// JNK cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// sma GTPase regulator activity /// ATP binding /// ATP binding /// kinase activity /// transferase activity
1569238_a_at	13.58	---	CDNA clone IMAGE:5259731	---	---	---
227868_at	13.55	LOC154761	hypothetical LOC154761	---	---	---
212397_at	13.28	RDX	radixin	---	cytoskeletal anchoring /// sensory perception of sound /// microvillus biogenesis /// apical protein localization /// barbed-end actin filament capping	actin binding /// structural molecule activity /// binding /// protein binding /// cytoskeletal protein binding
214059_at	12.98	IFI44	interferon-induced protein 44	---	response to virus	---
204917_s_at	12.79	MLLT3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3	---	transcription /// regulation of transcription, DNA-dependent	---
238846_at	12.11	TNFRSF11A	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	---	ossification /// immune response /// signal transduction /// cell-cell signaling /// multicellular organismal development /// sensory perception of sound /// positive regulation of cell proliferation /// lymph node development	receptor activity /// receptor activity /// protein binding /// protein binding
234967_at	11.91	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	---	immune response /// signal transduction /// cell surface receptor linked signal transduction	receptor activity /// receptor activity /// hematopoietin/interferon-class (D200-domain) cytokine receptor activity /// interleukin-6 receptor activity /// oncostatin-M receptor activity /// protein binding
201508_at	11.84	IGFBP4	insulin-like growth factor binding protein 4	Smooth_muscle_contraction	skeletal development /// regulation of cell growth /// DNA metabolic process /// inflammatory response /// signal transduction /// cell proliferation	insulin-like growth factor binding /// growth factor binding
225368_at	11.7	HIPK2	homeodomain interacting protein kinase 2	---	transcription /// regulation of transcription, DNA-dependent /// protein amino acid phosphorylation /// apoptosis /// induction of apoptosis by intracellular signals /// virus-host interaction /// positive regulation of transforming growth factor beta receptor signaling pathway /// positive regulation of JNK cascade	nucleotide binding /// transcription corepressor activity /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// protein binding /// ATP bindin /// kinase activity /// transferase activity /// virion binding
200952_s_at	11.69	CCND2	cyclin D2	Cell_cycle_KEGG /// G1_to_S_cell_cycle_Reactome /// Ovarian_Infertility_Genes /// Wnt_signaling	positive regulation of protein amino acid phosphorylation /// cell cycle /// positive regulation of cell proliferation /// positive regulation of cyclin-dependent protein kinase activity /// cell division	protein binding /// protein binding /// protein kinase binding /// protein kinase binding
235343_at	11.69	VASH2	vasohibin 2	---	---	---
201105_at	10.85	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	---	apoptosis /// regulation of apoptosis /// positive regulation of I-kappaB kinase/NF-kappaB cascade /// myoblast differentiation	signal transducer activity /// protein binding /// sugar binding
222392_x_at	10.85	PERP	PERP, TP53 apoptosis effector	---	apoptosis /// cell adhesion	structural molecule activity /// protein binding
226695_at	10.7	PRRX1	paired related homeobox 1	---	regulation of transcription, DNA-dependent /// multicellular organismal development /// regulation of transcription	DNA binding /// transcription factor activity /// transcription coactivator activity /// sequence-specific DNA binding
1562403_a_at	10.66	SLC8A3	solute carrier family 8 (sodium-calcium exchanger), member 3	Calcium_regulation_in_cardiac_cells	transport /// ion transport /// sodium ion transport /// calcium ion transport /// cell communication	calcium:sodium antiporter activity /// calcium ion binding /// calmodulin binding /// antiporter activity /// sodium ion binding
210479_s_at	10.03	RORA	RAR-related orphan receptor A	Nuclear_Receptors	transcription /// regulation of transcription, DNA-dependent /// signal transduction /// positive regulation of transcription from RNA polymerase II promoter	DNA binding /// transcription factor activity /// transcription facto activity /// steroid hormone receptor activity /// receptor activity /// ligand-dependent nuclear receptor activity /// ligand-dependent nuclear receptor activity /// protein binding /// zinc io binding /// sequence-specific DNA binding /// metal ion binding
211826_s_at	9.938	AFF1	AF4/FMR2 family, member 1	---	---	transcription factor activity
228365_at	9.855	CPNE8	copine VIII	---	---	---
231202_at	9.837	ALDH1L2	Aldehyde dehydrogenase 1 family, member L2	---	one-carbon compound metabolic process /// metabolic process /// biosynthetic process /// 10-formyltetrahydrofolate catabolic process	catalytic activity /// methyltransferase activity /// formyltetrahydrofolate dehydrogenase activity /// oxidoreductase activity /// hydroxymethyl-, formyl- and related transferase activity /// phosphopantetheine binding /// cofactor binding
209684_at	9.815	RIN2	Ras and Rab interactor 2	---	endocytosis /// signal transduction /// small GTPase mediated signal transduction	small GTPase regulator activity /// GTPase activator activity /// protein binding /// Rab guanyl-nucleotide exchange factor activity
224204_x_at	9.743	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// signal transduction /// entrainment of circadian clock /// regulation of transcription /// rhythmic process	DNA binding /// transcription factor activity /// signal transducer activity /// transcription regulator activity
201645_at	9.576	TNC	tenascin C (hexabrachion)	---	cell adhesion /// cell adhesion /// signal transduction /// neuromuscular junction development	receptor binding /// binding /// protein binding
205990_s_at	9.526	WNT5A	wingless-type MMTV integration site family, member 5A	Wnt_signaling	signal transduction /// Wnt receptor signaling pathway, calcium modulating pathway /// JNK cascade /// cell-cell signaling /// multicellular organismal development /// anatomical structure morphogenesis /// embryonic development /// Wnt receptor signaling pathway /// lung development /// embryonic limb morphogenesis	signal transducer activity /// signal transducer activity /// receptor binding
219654_at	9.401	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	---	protein amino acid dephosphorylation /// signal transduction /// multicellular organismal development /// dephosphorylation /// cellular protein metabolic process	protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// protein binding /// ATP binding /// phosphoric monoester hydrolase activity
227204_at	9.365	PARD6G	par-6 partitioning defective 6 homolog gamma (C. elegans)	---	cell cycle /// cell division	protein binding /// protein binding
221606_s_at	9.085	NSBP1	nucleosomal binding protein 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	DNA binding /// chromatin binding /// transcription activator activity
232210_at	8.929	---	CDNA FLJ14056 fis, clone HEMBB1000335	---	---	---
227561_at	8.635	DDR2	discoidin domain receptor tyrosine kinase 2	---	protein amino acid phosphorylation /// cell adhesion /// cell adhesion /// signal transduction /// transmembrane receptor protein tyrosine kinase signaling pathway /// positive regulation of cell proliferation	nucleotide binding /// protein tyrosine kinase activity /// transmembrane receptor protein tyrosine kinase activity /// transmembrane receptor protein tyrosine kinase activity /// receptor activity /// ATP binding /// kinase activity /// transferase activity
204864_s_at	8.619	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	---	immune response /// signal transduction /// cell surface receptor linked signal transduction	receptor activity /// receptor activity /// hematopoietin/interferon-class (D200-domain) cytokine receptor activity /// interleukin-6 receptor activity /// oncostatin-M receptor activity /// protein binding
238395_at	8.609	---	Transcribed locus	---	---	---
220567_at	8.569	IKZF2	IKAROS family zinc finger 2 (Helios)	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
213865_at	8.527	DCBLD2	discoidin, CUB and LCCL domain containing 2	---	cell adhesion /// negative regulation of cell growth /// negative regulation of cell growth /// intracellular receptor-mediated signaling pathway /// wound healing /// wound healing	protein binding
235846_at	8.382	---	CDNA FLJ23692 fis, clone HEP10227	---	---	---
201309_x_at	8.288	C5orf13	chromosome 5 open reading frame 13	---	regulation of transforming growth factor beta receptor signaling pathway	protein binding
204044_at	8.05	QPRT	quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating))	---	metabolic process /// NAD biosynthetic process /// pyridine nucleotide biosynthetic process /// quinolinate catabolic process /// quinolinate metabolic process /// protein oligomerization	catalytic activity /// nicotinate-nucleotide diphosphorylase (carboxylating) activity /// nicotinate-nucleotide diphosphorylase (carboxylating) activity /// hormone activity /// transferase activity /// transferase activity, transferring glycosyl groups /// protein homodimerization activity
1569361_at	7.791	7A5	putative binding protein 7a5	---	---	---
229070_at	7.712	C6orf105	chromosome 6 open reading frame 105	---	---	---
211078_s_at	7.674	STK3	serine/threonine kinase 3 (STE20 homolog, yeast)	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// apoptosis /// signal transduction /// signal transduction /// protein kinase cascade /// positive regulation of apoptosis	nucleotide binding /// magnesium ion binding /// magnesium ion binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein binding /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity /// metal ion binding /// protein dimerization activity

223449_at	7.662	SEMA6A	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	---	apoptosis /// cytoskeleton organization and biogenesis /// cell surface receptor linked signal transduction /// multicellular organismal development /// nervous system development /// nervous system development /// axon guidance /// organ morphogenesis /// cell differentiation	receptor activity /// protein binding
37966_at	7.653	PARVB	parvin, beta	---	cell adhesion	actin binding /// protein binding /// protein binding
213455_at	7.552	FAM114A1	family with sequence similarity 114, member A1	---	---	---
201301_s_at	7.463	ANXA4	annexin A4	Prostaglandin_synthesis_regulation	anti-apoptosis /// signal transduction /// negative regulation of coagulation	phospholipase inhibitor activity /// calcium ion binding /// calcium ion binding /// calcium-dependent phospholipid binding /// calcium-dependent phospholipid binding
211000_s_at	7.458	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	---	immune response /// signal transduction /// cell surface receptor linked signal transduction	receptor activity /// receptor activity /// hematopoietin/interferon-class (D200-domain) cytokine receptor activity /// interleukin-6 receptor activity /// oncostatin-M receptor activity /// protein binding
224560_at	7.376	TIMP2	TIMP metallopeptidase inhibitor 2	Matrix_Metalloproteinases	negative regulation of cell proliferation /// regulation of cAMP metabolic process /// regulation of MAPKKK cascade /// regulation of neuron differentiation	enzyme inhibitor activity /// integrin binding /// protein binding /// protein binding /// enzyme activator activity /// metalloendopeptidase inhibitor activity /// metalloendopeptidase inhibitor activity
39402_at	7.273	IL1B	interleukin 1, beta	Smooth_muscle_contraction	activation of MAPK activity /// angiogenesis /// fever /// positive regulation of protein amino acid phosphorylation /// apoptosis /// anti-apoptosis /// inflammatory response /// inflammatory response /// inflammatory response /// immune response /// signal transduction /// elevation of cytosolic calcium ion concentration /// cell-cell signaling /// cell proliferation /// negative regulation of cell proliferation /// cytokine and chemokine mediated signaling pathway /// cytokine and chemokine mediated signaling pathway /// neutrophil chemotaxis /// positive regulation of interleukin-6 production /// regulation of I-kappaB kinase/NF-kappaB cascade /// positive regulation of I-kappaB kinase/NF-kappaB cascade /// positive regulation of chemokine biosynthetic process /// positive regulation of interleukin-6 biosynthetic process /// positive regulation of JNK cascade /// leukocyte migration /// positive regulation of transcription factor activity	signal transducer activity /// cytokine activity /// cytokine activity /// cytokine activity /// interleukin-1 receptor binding /// interleukin-1 receptor binding /// protein binding /// growth factor activity
228266_s_at	7.27	HDGFRP3	hepatoma-derived growth factor, related protein 3	---	cell proliferation	growth factor activity
239315_at	7.249	FAM139A	family with sequence similarity 139, member A	---	---	---
1553781_at	7.235	ZC3HAV1L	zinc finger CCCH-type, antiviral 1-like	---	---	---
224911_s_at	7.194	DCBLD2	discoidin, CUB and LCCL domain containing 2	---	cell adhesion /// negative regulation of cell growth /// negative regulation of cell growth /// intracellular receptor-mediated signaling pathway /// wound healing /// wound healing	protein binding
242783_at	7.171	---	Transcribed locus	---	---	---
218589_at	7.137	P2RY5	purinergic receptor P2Y, G-protein coupled, 5	GPCRDB_Class_A_Rhodopsin-like /// Nucleotide_GPCRs	signal transduction /// G-protein coupled receptor protein signaling pathway /// G-protein coupled receptor protein signaling pathway	rhodopsin-like receptor activity /// signal transducer activity /// receptor activity /// G-protein coupled receptor activity /// G-protein coupled receptor activity /// purinergic nucleotide receptor activity, G-protein coupled
1558647_at	7.114	FLJ46481 /// SH3D19	SH3 domain containing 19 /// FLJ46481 protein	---	---	---
213425_at	7.054	WNT5A	wingless-type MMTV integration site family, member 5A	Wnt_signaling	signal transduction /// Wnt receptor signaling pathway, calcium modulating pathway /// JNK cascade /// cell-cell signaling /// multicellular organismal development /// anatomical structure morphogenesis /// embryonic development /// Wnt receptor signaling pathway /// lung development /// embryonic limb morphogenesis	signal transducer activity /// signal transducer activity /// receptor binding
211706_s_at	6.944	SCD	stearoyl-CoA desaturase (delta-9-desaturase)	Fatty_Acid_Synthesis	lipid metabolic process /// fatty acid biosynthetic process /// lipid biosynthetic process	stearoyl-CoA 9-desaturase activity /// stearoyl-CoA 9-desaturase activity /// iron ion binding /// oxidoreductase activity /// oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water
205816_at	6.928	ITGB8	integrin, beta 8	Integrin-mediated_cell_adhesion_KEGG	ganglioside metabolic process /// cell adhesion /// cell adhesion /// cell-matrix adhesion /// integrin-mediated signaling pathway /// multicellular organismal development	receptor activity /// binding /// protein binding
1556964_s_at	6.843	---	CDNA FLJ34002 fis, clone FCBBF1000206	---	---	---
203773_x_at	6.774	BLVRA	biliverdin reductase A	---	metabolic process /// heme catabolic process /// oxidation reduction	catalytic activity /// biliverdin reductase activity /// biliverdin reductase activity /// binding /// zinc ion binding /// oxidoreductase activity /// metal ion binding
202022_at	6.771	ALDOC	aldolase C, fructose-bisphosphate	Glycolysis_and_Gluconeogenesis	fructose metabolic process /// glycolysis /// microtubule-based process /// microtubule-based movement /// metabolic process /// protein polymerization	nucleotide binding /// catalytic activity /// GTPase activity /// fructose-bisphosphate aldolase activity /// fructose-bisphosphate aldolase activity /// structural molecule activity /// protein binding /// GTP binding /// lyase activity
236678_at	6.749	---	Transcribed locus	---	---	---
1405_i_at	6.728	CCL5	chemokine (C-C motif) ligand 5	---	cellular calcium ion homeostasis /// exocytosis /// cell motility /// chemotaxis /// chemotaxis /// inflammatory response /// inflammatory response /// immune response /// immune response /// cellular defense response /// response to oxidative stress /// cell adhesion /// signal transduction /// cell-cell signaling /// response to virus /// negative regulation of viral genome replication	signal transducer activity /// receptor binding /// cytokine activity /// chemokine activity /// chemokine activity /// chemoattractant activity
216996_s_at	6.664	IRF4	interferon regulatory factor 4	Apoptosis	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// T cell activation /// positive regulation of interleukin-10 biosynthetic process /// positive regulation of interleukin-10 biosynthetic process /// positive regulation of interleukin-2 biosynthetic process /// positive regulation of interleukin-2 biosynthetic process /// positive regulation of interleukin-13 biosynthetic process /// positive regulation of interleukin-13 biosynthetic process /// positive regulation of interleukin-4 biosynthetic process /// positive regulation of interleukin-4 biosynthetic process /// regulation of T-helper cell differentiation /// positive regulation of transcription /// positive regulation of transcription	DNA binding /// transcription factor activity /// transcription factor activity /// RNA polymerase II transcription factor activity /// transcription factor binding /// transcription activator activity /// transcription activator activity
231579_s_at	6.6	TIMP2	TIMP metallopeptidase inhibitor 2	Matrix_Metalloproteinases	negative regulation of cell proliferation /// regulation of cAMP metabolic process /// regulation of MAPKKK cascade /// regulation of neuron differentiation	enzyme inhibitor activity /// integrin binding /// protein binding /// protein binding /// enzyme activator activity /// metalloendopeptidase inhibitor activity /// metalloendopeptidase inhibitor activity
1561882_at	6.553	SYTL3	synaptotagmin-like 3	---	intracellular protein transport	protein binding /// zinc ion binding /// Rab GTPase binding
204451_at	6.544	FZD1	frizzled homolog 1 (Drosophila)	Wnt_signaling	signal transduction /// cell surface receptor linked signal transduction /// G-protein coupled receptor protein signaling pathway /// cell-cell signaling /// multicellular organismal development /// Wnt receptor signaling pathway /// negative regulation of transcription /// epithelial cell differentiation	signal transducer activity /// receptor activity /// transmembrane receptor activity /// non-G-protein coupled 7TM receptor activity /// G-protein coupled receptor activity /// protein binding
202555_s_at	6.531	MYLK	myosin light chain kinase	---	protein amino acid phosphorylation /// protein amino acid phosphorylation	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// myosin light chain kinase activity /// myosin light chain kinase activity /// signal transducer activity /// calcium ion binding /// calmodulin binding /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
216048_s_at	6.422	RHOBTB3	Rho-related BTB domain containing 3	---	protein localization	GTPase activity /// protein binding
235463_s_at	6.204	LASS6	LAG1 homolog, ceramide synthase 6	---	regulation of transcription, DNA-dependent /// lipid biosynthetic process	DNA binding /// transcription factor activity /// sequence-specific DNA binding
1569024_at	6.196	FAM13A1	family with sequence similarity 13, member A1	---	signal transduction	---
230175_s_at	6.073	---	---	---	---	---
220942_x_at	6.039	C3orf28	chromosome 3 open reading frame 28	---	---	---
229109_s_at	6.003	BLVRA	Biliverdin reductase A	---	metabolic process /// heme catabolic process /// oxidation reduction	catalytic activity /// biliverdin reductase activity /// biliverdin reductase activity /// binding /// zinc ion binding /// oxidoreductase activity /// metal ion binding
232151_at	5.971	7A5	putative binding protein 7a5	---	---	---
1561167_at	5.827	---	Full length insert cDNA clone YA75A09	---	---	---

226905_at	5.741	FAM101B	family with sequence similarity 101, member B	---	---	---
204457_s_at	5.639	GAS1	growth arrest-specific 1	---	cell cycle /// cell cycle arrest /// cell cycle arrest /// negative regulation of cell proliferation /// programmed cell death /// cell fate commitment /// negative regulation of S phase of mitotic cell cycle /// eye morphogenesis /// negative regulation of epithelial cell proliferation	protein binding
217419_x_at	5.584	AGRN	agrin	---	signal transduction /// acetylcholine receptor signaling, muscarinic pathway /// acetylcholine receptor signaling, muscarinic pathway /// synaptic transmission /// receptor clustering /// receptor clustering /// receptor clustering /// clustering of voltage-gated sodium channels /// synapse organization and biogenesis	structural constituent of cytoskeleton /// calcium ion binding /// protein binding /// protein binding /// laminin binding /// laminin binding
243275_at	5.546	---	---	---	---	---
208999_at	5.538	Sep 08	septin 8	---	cell cycle	nucleotide binding /// protein binding /// protein binding /// GTP binding
213324_at	5.437	SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	Integrin-mediated_cell_adhesion_KEGG	protein amino acid phosphorylation /// signal transduction /// signal complex assembly /// protein kinase cascade	nucleotide binding /// protein kinase activity /// protein kinase activity /// protein tyrosine kinase activity /// protein tyrosine kinase activity /// non-membrane spanning protein tyrosine kinase activity /// SH3/SH2 adaptor activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity /// SH2 domain binding
242092_at	5.428	---	---	---	---	---
203790_s_at	5.354	HRSP12	heat-responsive protein 12	---	regulation of translational termination	nuclease activity /// endonuclease activity /// hydrolase activity
220569_at	5.24	---	---	---	---	---
1570021_at	5.217	LOC360030	homeobox C14	---	regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// sequence-specific DNA binding
209290_s_at	5.039	NFIB	nuclear factor I/B	---	DNA replication /// transcription /// regulation of transcription, DNA-dependent	DNA binding /// transcription factor activity /// transcription factor activity
201468_s_at	4.956	NQO1	NAD(P)H dehydrogenase, quinone 1	---	xenobiotic metabolic process /// nitric oxide biosynthetic process /// synaptic transmission, cholinergic /// response to toxin	NAD(P)H dehydrogenase (quinone) activity /// NAD(P)H dehydrogenase (quinone) activity /// cytochrome-b5 reductase activity /// electron carrier activity /// oxidoreductase activity /// coenzyme binding
226099_at	4.871	ELL2	elongation factor, RNA polymerase II, 2	---	transcription /// regulation of transcription, DNA-dependent /// RNA elongation from RNA polymerase II promoter	RNA polymerase II transcription factor activity /// translation elongation factor activity
204747_at	4.829	IFIT3	interferon-induced protein with tetratricopeptide repeats 3	---	---	binding
1565635_at	4.821	---	CDNA: FLJ20875 fis, clone ADKA02835	---	---	---
220183_s_at	4.792	NUDT6	nudix (nucleoside diphosphate linked moiety X)-type motif 6	---	---	growth factor activity /// hydrolase activity
204201_s_at	4.71	PTPN13	protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)	Apoptosis_KEGG	protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// dephosphorylation	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// non-membrane spanning protein tyrosine phosphatase activity /// receptor activity /// structural molecule activity /// binding /// protein binding /// protein binding /// hydrolase activity /// phosphoric monoester hydrolase activity
209879_at	4.536	SELPLG	selectin P ligand	---	cell adhesion /// cell adhesion	receptor binding /// protein binding /// sugar binding /// bacterial binding
1555626_a_at	4.434	SLAMF1	signaling lymphocytic activation molecule family member 1	---	positive regulation of cell proliferation /// lymphocyte activation	antigen binding /// receptor activity /// transmembrane receptor activity
1554452_a_at	4.427	HIG2	hypoxia-inducible protein 2	---	response to stress /// response to stress	---
1556325_at	4.418	FILIP1	filamin A interacting protein 1	---	---	---
1552373_s_at	4.394	C4orf33	chromosome 4 open reading frame 33	---	---	---
207095_at	4.371	SLC10A2	solute carrier family 10 (sodium/bile acid cotransporter family), member 2	---	transport /// transport /// ion transport /// sodium ion transport /// organic anion transport /// bile acid and bile salt transport	bile acid:sodium symporter activity /// bile acid:sodium symporter activity /// symporter activity /// sodium ion binding
215100_at	4.357	C6orf105	chromosome 6 open reading frame 105	---	---	---
37965_at	4.327	PARVB	parvin, beta	---	cell adhesion	actin binding /// protein binding /// protein binding
231092_s_at	4.326	ZNRF1	Zinc and ring finger 1	---	ubiquitin cycle	protein binding /// zinc ion binding /// ligase activity /// metal ion binding
225450_at	4.238	AMOTL1	angiomotin like 1	---	---	protein binding /// identical protein binding
1566766_a_at	4.209	7A5	putative binding protein 7a5	---	---	---
240113_at	4.208	---	Transcribed locus	---	---	---
212446_s_at	4.176	LASS6	LAG1 homolog, ceramide synthase 6	---	regulation of transcription, DNA-dependent /// lipid biosynthetic process	DNA binding /// transcription factor activity /// sequence-specific DNA binding
216980_s_at	4.141	SPN	sialophorin (leukosialin, CD43)	---	response to protozoan /// negative regulation of type IV hypersensitivity /// chemotaxis /// immune response /// cellular defense response /// negative regulation of cell adhesion /// negative regulation of cell adhesion /// establishment and/or maintenance of cell polarity /// signal transduction /// cell surface receptor linked signal transduction /// induction of apoptosis by extracellular signals /// T cell costimulation /// positive regulation of T cell proliferation /// negative regulation of T cell proliferation /// positive regulation of tumor necrosis factor biosynthetic process /// positive regulation of tumor necrosis factor biosynthetic process /// defense response to bacterium /// defense response to bacterium /// negative thymic T cell selection /// regulation of defense response to virus /// regulation of immune response	transmembrane receptor activity /// binding /// protein binding /// bacterial binding /// bacterial binding
211793_s_at	4.046	ABI2	abl interactor 2	---	cell motility /// cytoskeleton organization and biogenesis /// actin polymerization and/or depolymerization /// cell migration /// peptidyl-tyrosine phosphorylation	DNA binding /// SH3/SH2 adaptor activity /// cytoskeletal adaptor activity /// SH3 domain binding /// kinase binding
242724_x_at	4	---	---	---	---	---
222237_s_at	3.982	ZNF228	zinc finger protein 228	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
237940_s_at	3.975	---	Transcribed locus	---	---	---
226010_at	3.879	SLC25A23	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23	---	transport	transporter activity /// binding /// calcium ion binding
235281_x_at	3.871	AHNAK	AHNAK nucleoprotein	---	nervous system development	nucleic acid binding /// protein binding
1560680_at	3.844	---	Transcribed locus, moderately similar to NP_001026424.1 heterogeneous nuclear ribonucleoprotein A3 [Gallus gallus]	---	---	---
209920_at	3.815	BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase)	---	skeletal development /// protein amino acid phosphorylation /// transmembrane receptor protein serine/threonine kinase signaling pathway /// transmembrane receptor protein serine/threonine kinase signaling pathway /// regulation of cell proliferation	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// transmembrane receptor protein serine/threonine kinase activity /// receptor signaling protein serine/threonine kinase activity /// receptor activity /// transforming growth factor beta receptor activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity /// manganese ion binding /// metal ion binding
211071_s_at	3.752	MLLT11	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 11	---	---	---
229440_at	3.751	RBM47	RNA binding motif protein 47	---	---	nucleic acid binding /// RNA binding
209615_s_at	3.734	PAK1	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	Integrin-mediated_cell_adhesion_KEGG	MAPKKK cascade /// protein complex assembly /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// apoptosis /// ER-nuclear signaling pathway /// cytoskeleton organization and biogenesis /// actin polymerization and/or depolymerization /// positive regulation of JNK activity /// protein amino acid autophosphorylation	nucleotide binding /// catalytic activity /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// small GTPase regulator activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity
241705_at	3.722	ABCA5	ATP-binding cassette, sub-family A (ABC1), member 5	---	transport	nucleotide binding /// ATP binding /// ATPase activity /// nucleoside-triphosphatase activity
218964_at	3.717	ARID3B	AT rich interactive domain 3B (BRIGHT-like)	---	transcription /// regulation of transcription, DNA-dependent	DNA binding /// DNA binding
204082_at	3.671	PBX3	pre-B-cell leukemia homeobox 3	---	transcription /// regulation of transcription, DNA-dependent /// anterior compartment specification /// posterior compartment specification /// regulation of transcription	DNA binding /// transcription factor activity /// sequence-specific DNA binding
201061_s_at	3.581	STOM	stomatin	---	protein homooligomerization	protein binding

202862_at	3.557	FAH	fumarylacetoacetate hydrolase (fumarylacetoacetase)	---	arginine catabolic process /// L-phenylalanine catabolic process /// tyrosine catabolic process /// tyrosine catabolic process /// metabolic process /// aromatic amino acid family metabolic process	magnesium ion binding /// catalytic activity /// fumarylacetoacetase activity /// fumarylacetoacetase activity /// calcium ion binding /// hydrolase activity /// metal ion binding
239630_at	3.547	---	---	---	---	---
1560316_s_at	3.544	GLCCI1	glucocorticoid induced transcript 1	---	---	protein binding
238409_x_at	3.511	OXR1	oxidation resistance 1	---	response to stress /// response to oxidative stress /// cell wall catabolic process	---
230066_at	3.47	SNX25	sorting nexin 25	---	transport /// cell communication /// intracellular signaling cascade /// protein transport	signal transducer activity /// protein binding /// phosphoinositide binding
1554997_a_at	3.461	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	Eicosanoid_Synthesis /// Prostaglandin_synthesis_regulation	prostaglandin biosynthetic process /// fatty acid biosynthetic process /// prostaglandin metabolic process /// cell motility /// inflammatory response /// response to oxidative stress /// regulation of blood pressure /// regulation of blood pressure /// negative regulation of cell proliferation /// lipid biosynthetic process /// cyclooxygenase pathway /// anagen /// regulation of inflammatory response /// oxidation reduction	peroxidase activity /// peroxidase activity /// prostaglandin-endoperoxide synthase activity /// prostaglandin-endoperoxide synthase activity /// prostaglandin-endoperoxide synthase activity /// iron ion binding /// protein binding /// electron donor activity /// oxidoreductase activity /// oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen /// heme binding /// metal ion binding
213714_at	3.431	CACNB2	calcium channel, voltage-dependent, beta 2 subunit	---	transport /// transport /// ion transport /// calcium ion transport /// calcium ion transport /// neuromuscular junction development	ion channel activity /// voltage-gated ion channel activity /// voltage-gated calcium channel activity /// voltage-gated calcium channel activity /// calcium channel activity /// calcium channel activity /// calcium ion binding
1569703_a_at	3.42	---	Transcribed locus	---	---	---
239310_at	3.413	---	Transcribed locus	---	---	---
1553995_a_at	3.395	NT5E	5'-nucleotidase, ecto (CD73)	---	DNA metabolic process /// nucleotide catabolic process	nucleotide binding /// 5'-nucleotidase activity /// 5'-nucleotidase activity /// zinc ion binding /// hydrolase activity /// hydrolase activity, acting on ester bonds /// metal ion binding
239857_at	3.324	---	CDNA FLJ37227 fis, clone BRAMY2000277	---	---	---
223830_s_at	3.304	TRIM5	tripartite motif-containing 5	---	ubiquitin cycle /// response to virus	protein binding /// protein binding /// zinc ion binding /// ligase activity /// metal ion binding
1556601_a_at	3.298	SPATA13	Spermatogenesis associated 13	---	regulation of Rho protein signal transduction	Rho guanyl-nucleotide exchange factor activity
244014_x_at	3.278	---	Transcribed locus	---	---	---
1565889_at	3.262	---	Full length insert cDNA YQ03C01	---	---	---
208116_s_at	3.259	MAN1A1	mannosidase, alpha, class 1A, member 1	---	protein amino acid glycosylation /// metabolic process	mannosyl-oligosaccharide 1,2-alpha-mannosidase activity /// mannosyl-oligosaccharide 1,2-alpha-mannosidase activity /// calcium ion binding /// calcium ion binding /// mannosidase activity /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds
226407_at	3.254	---	CDNA FLJ30519 fis, clone BRAWH2000859	---	---	---
213914_s_at	3.250	SPTDN1	Spectrin, beta, non-erythrocytic 1	---	barbed-end actin filament capping	actin binding /// actin binding /// structural constituent of cytoskeleton /// structural constituent of cytoskeleton /// protein binding /// calmodulin binding
205211_s_at	3.229	RIN1	Ras and Rab interactor 1	---	endocytosis /// signal transduction /// signal transduction	GTPase activator activity /// protein binding /// protein binding /// protein binding
1556682_s_at	3.209	---	Full length insert cDNA clone ZD73H04	---	---	---
207524_at	3.164	ST7	suppression of tumorigenicity 7	---	---	---
202722_s_at	3.162	GFPT1	glutamine-fructose-6-phosphate transaminase 1	---	carbohydrate metabolic process /// fructose 6-phosphate metabolic process /// energy reserve metabolic process /// glutamine metabolic process /// metabolic process /// carbohydrate biosynthetic process	glutamine-fructose-6-phosphate transaminase (isomerizing) activity /// glutamine-fructose-6-phosphate transaminase (isomerizing) activity /// sugar binding /// transaminase activity /// transferase activity
AFFX-HUMISGF3A/M97935_5_at	3.074	STAT1	signal transducer and activator of transcription 1, 91kDa	---	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// caspase activation /// signal transduction /// signal transduction /// I-kappaB kinase/NF-kappaB cascade /// JAK-STAT cascade /// tyrosine phosphorylation of STAT protein /// STAT protein nuclear translocation /// response to virus /// regulation of transcription	DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// signal transducer activity /// hematopoietin/interferon-class (D200-domain) cytokine receptor signal transducer activity /// calcium ion binding /// protein binding /// protein binding
227921_at	3.049	---	---	---	---	---
232230_at	3.025	C10orf75	Chromosome 10 open reading frame 75	---	---	---
224916_at	3.008	TMEM173	transmembrane protein 173	---	---	---
234196_at	3.004	---	CDNA: FLJ21377 fis, clone COL03255	---	---	---
226001_at	3	RAB3D	RAB3D, member RAS oncogene family	---	transport /// exocytosis /// small GTPase mediated signal transduction /// hemocyte development /// protein transport /// regulation of exocytosis	nucleotide binding /// GTPase activity /// protein binding /// protein binding /// GTP binding
233957_at	2.99	---	MRNA; cDNA DKFZp566M223 (from clone DKFZp566M223)	---	---	---
216428_x_at	2.98	KIR3DX1	killer cell immunoglobulin-like receptor, three domains, X1	---	---	---
1560509_at	2.951	---	MRNA; cDNA DKFZp547H194 (from clone DKFZp547H194)	---	---	---
219951_s_at	2.904	C20orf12	chromosome 20 open reading frame 12	---	---	zinc ion binding
203299_s_at	2.875	AP1S2	adaptor-related protein complex 1, sigma 2 subunit	---	protein complex assembly /// transport /// intracellular protein transport /// endocytosis /// protein transport /// vesicle-mediated transport	protein binding
241502_x_at	2.869	---	Transcribed locus	---	---	---
1562208_a_at	2.867	---	MRNA; cDNA DKFZp761E11121 (from clone DKFZp761E11121)	---	---	---
232987_at	2.818	ARL17	ADP-ribosylation factor-like 17	---	transport /// protein transport /// vesicle-mediated transport	nucleotide binding /// GTP binding
225792_at	2.795	HOOK1	hook homolog 1 (Drosophila)	---	microtubule cytoskeleton organization and biogenesis /// multicellular organismal development /// spermatogenesis /// cell differentiation	protein binding /// microtubule binding
207268_x_at	2.77	ABI2	abl interactor 2	---	cell motility /// cytoskeleton organization and biogenesis /// actin polymerization and/or depolymerization /// cell migration /// peptidyl-tyrosine phosphorylation	DNA binding /// SH3/SH2 adaptor activity /// cytoskeletal adaptor activity /// SH3 domain binding /// kinase binding
229861_at	2.752	---	MRNA; cDNA DKFZp547K189 (from clone DKFZp547K189)	---	---	---
1569499_at	2.738	---	CDNA clone IMAGE:3840913	---	---	---
232682_at	2.732	MREG	melanoregulin	---	melanocyte differentiation /// anagen /// pigmentation during development	---
1554106_at	2.702	ALS2CR16	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 16	---	---	---
243827_at	2.699	---	Transcribed locus	---	---	---
1556081_at	2.67	---	CDNA FLJ33441 fis, clone BRACE2021932	---	---	---
216794_at	2.641	---	CDNA: FLJ23203 fis, clone ADKA02487	---	---	---
222966_at	2.565	---	---	---	---	---
233255_s_at	2.562	BIVM	basic, immunoglobulin-like variable motif containing	---	---	---
239385_at	2.56	TFG	TRK-fused gene	Apoptosis_KEGG	protein amino acid phosphorylation /// transmembrane receptor protein tyrosine kinase signaling pathway /// positive regulation of I-kappaB kinase/NF-kappaB cascade	nucleotide binding /// transmembrane receptor protein tyrosine kinase activity /// signal transducer activity /// receptor activity /// protein binding /// ATP binding /// transferase activity /// identical protein binding
222351_at	2.559	PPP2R1B	protein phosphatase 2 (formerly 2A), regulatory subunit A, beta isoform	Glycogen_Metabolism	---	antigen binding /// protein serine/threonine phosphatase activity /// binding /// protein binding /// protein binding /// protein heterodimerization activity
244047_at	2.557	---	Transcribed locus	---	---	---
1566951_at	2.553	---	AD00y mRNA sequence	---	---	---
215213_at	2.525	NUP54	nucleoporin 54kDa	---	transport /// protein transport /// mRNA transport /// intracellular protein transport across a membrane	---
207143_at	2.519	CDK6	cyclin-dependent kinase 6	---	G1 phase of mitotic cell cycle /// positive regulation of cell-matrix adhesion /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// cell cycle /// regulation of gene expression /// regulation of gene expression /// gliogenesis /// cell dedifferentiation /// regulation of erythrocyte differentiation /// negative regulation of osteoblast differentiation /// positive regulation of fibroblast proliferation /// negative regulation of epithelial cell proliferation /// cell division	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// cyclin-dependent protein kinase activity /// cyclin-dependent protein kinase activity /// protein binding /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity /// cyclin binding
243736_at	2.516	---	Transcribed locus	---	---	---
228360_at	2.5	LOC130576	hypothetical protein LOC130576	---	---	---

240152_at	2.468	---	---	---	---	---
206385_at	2.437	MDM2	Mdm2 p53 binding protein homolog (mouse)	Apoptosis /// Apoptosis_GenMAPP /// Cell_cycle_KEGG /// G1_to_S_cell_cycle_Reactome	negative regulation of transcription from RNA polymerase II promoter /// negative regulation of transcription from RNA polymerase II promoter /// protein complex assembly /// ubiquitin cycle /// negative regulation of cell proliferation /// protein ubiquitination /// regulation of protein catabolic process	ubiquitin-protein ligase activity /// protein binding /// protein binding /// protein binding /// zinc ion binding /// zinc ion binding /// ligase activity /// negative regulator of basal transcription activity /// negative regulator of basal transcription activity /// enzyme binding /// identical protein binding /// metal ion binding
212979_s_at	2.421	FAM115A	family with sequence similarity 115, member A	---	---	---
207744_at	2.396	---	---	---	---	---
215436_at	2.396	HSDL2	Hydroxysteroid dehydrogenase like 2	---	metabolic process	catalytic activity /// binding /// sterol carrier activity /// oxidoreductase activity
209398_at	2.387	HIST1H1C	histone cluster 1, H1c	---	nucleosome assembly /// nucleosome assembly /// nucleosome positioning	DNA binding /// DNA binding /// protein binding
201940_at	2.384	CPD	carboxypeptidase D	---	proteolysis	carboxypeptidase activity /// metallocarboxypeptidase activity /// carboxypeptidase A activity /// carboxypeptidase D activity /// carboxypeptidase D activity /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// metallocarboxypeptidase D activity /// hydrolase activity /// metal ion binding
230733_at	2.379	---	Transcribed locus	---	---	---
1552788_a_at	2.37	HELB	helicase (DNA) B	---	DNA replication /// DNA replication, synthesis of RNA primer	helicase activity /// single-stranded DNA-dependent ATP-dependent DNA helicase activity /// ATP-dependent 5'-3' DNA helicase activity
243674_at	2.37	LOC100130623 /// RP11-160N1.10	hypothetical LOC401522 /// hypothetical protein LOC100130623	---	---	---
211914_x_at	2.351	NF1	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	---	MAPKKK cascade /// osteoblast differentiation /// metanephros development /// response to hypoxia /// liver development /// negative regulation of endothelial cell proliferation /// regulation of cell-matrix adhesion /// negative regulation of protein kinase activity /// cell cycle /// cell communication /// signal transduction /// Ras protein signal transduction /// negative regulation of neuroblast proliferation /// brain development /// peripheral nervous system development /// heart development /// visual learning /// Schwann cell development /// phosphoinositide 3-kinase cascade /// spinal cord development /// forebrain astrocyte development /// cerebral cortex development /// myelination in the peripheral nervous system /// actin cytoskeleton organization and biogenesis /// extracellular matrix organization and biogenesis /// collagen fibril organization /// adrenal gland development /// negative regulation of cell migration /// regulation of Ras GTPase activity /// positive regulation of Ras GTPase activity /// positive regulation of Ras GTPase activity /// wound healing /// negative regulation of trans-	GTPase activator activity /// Ras GTPase activator activity /// protein binding
229492_at	2.346	VANGL1	vang-like 1 (van gogh, Drosophila)	---	multicellular organismal development	protein binding /// protein binding
212256_at	2.314	GALNT10	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (GalNAc-T10)	---	---	polypeptide N-acetylgalactosaminyltransferase activity /// calcium ion binding /// sugar binding /// transferase activity /// transferase activity, transferring glycosyl groups /// manganese ion binding /// metal ion binding
212665_at	2.289	TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	---	protein amino acid ADP-ribosylation	nucleic acid binding /// NAD+ ADP-ribosyltransferase activity /// protein binding /// zinc ion binding /// transferase activity /// transferase activity, transferring glycosyl groups /// metal ion binding
219403_s_at	2.288	HPSE	heparanase	---	proteoglycan metabolic process /// inflammatory response	magnesium ion binding /// beta-glucuronidase activity /// calcium ion binding /// hydrolase activity
228157_at	2.277	ZNF207	zinc finger protein 207	---	regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// zinc ion binding /// zinc ion binding /// metal ion binding
211866_x_at	2.262	HFE	hemochromatosis	---	antigen processing and presentation of peptide antigen via MHC class I /// protein complex assembly /// transport /// ion transport /// iron ion transport /// iron ion transport /// cellular iron ion homeostasis /// receptor-mediated endocytosis /// immune response /// antigen processing and presentation	iron ion binding
213623_at	2.255	KIF3A	kinesin family member 3A	---	organelle organization and biogenesis /// microtubule-based movement	nucleotide binding /// motor activity /// microtubule motor activity /// protein binding /// ATP binding
234989_at	2.221	TncRNA	trophoblast-derived noncoding RNA	---	---	---
209170_s_at	2.174	GPM6B	glycoprotein M6B	---	multicellular organismal development /// nervous system development /// nervous system development /// cell differentiation	---
211137_s_at	2.146	ATP2C1	ATPase, Ca++ transporting, type 2C, member 1	---	transport /// ion transport /// cation transport /// calcium ion transport /// calcium ion transport /// calcium ion transport /// cellular calcium ion homeostasis /// cellular calcium ion homeostasis /// metabolic process /// epidermis development /// proton transport /// calcium-dependent cell-cell adhesion /// actin cytoskeleton reorganization /// Golgi calcium ion homeostasis /// Golgi calcium ion transport /// positive regulation of I-kappaB kinase/NF-kappaB cascade	nucleotide binding /// magnesium ion binding /// catalytic activity /// signal transducer activity /// calcium-transporting ATPase activity /// calcium-transporting ATPase activity /// calcium-transporting ATPase activity /// calcium ion binding /// ATP binding /// ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism /// hydrolase activity /// hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances /// metal ion binding
206386_s_at	2.137	MDM2	Mdm2 p53 binding protein homolog (mouse)	Apoptosis /// Apoptosis_GenMAPP /// Cell_cycle_KEGG /// G1_to_S_cell_cycle_Reactome	negative regulation of transcription from RNA polymerase II promoter /// negative regulation of transcription from RNA polymerase II promoter /// protein complex assembly /// ubiquitin cycle /// negative regulation of cell proliferation /// protein ubiquitination /// regulation of protein catabolic process	ubiquitin-protein ligase activity /// protein binding /// protein binding /// protein binding /// zinc ion binding /// zinc ion binding /// ligase activity /// negative regulator of basal transcription activity /// negative regulator of basal transcription activity /// enzyme binding /// identical protein binding /// metal ion binding
218856_at	2.134	TNFRSF21	tumor necrosis factor receptor superfamily, member 21	Apoptosis	apoptosis /// signal transduction	receptor activity /// protein binding
244067_x_at	2.134	---	Transcribed locus	---	---	---
1562644_at	2.127	---	MRNA; cDNA DKFZp566P2324 (from clone DKFZp566P2324)	---	---	---
1566896_at	2.096	---	Transcribed locus	---	---	---
1557944_s_at	2.081	CTNND1	catenin (cadherin-associated protein), delta 1	---	transcription /// regulation of transcription, DNA-dependent /// cell adhesion /// cell adhesion /// Wnt receptor signaling pathway /// cell-cell adhesion /// cell redox homeostasis	structural molecule activity /// binding /// protein binding
240964_at	2.07	---	Transcribed locus	---	---	---
211464_x_at	2.066	CASP6	caspase 6, apoptosis-related cysteine peptidase	Apoptosis /// Apoptosis_GenMAPP /// Apoptosis_KEGG	proteolysis /// proteolysis /// proteolysis /// apoptosis /// induction of apoptosis	protein binding /// peptidase activity /// cysteine-type peptidase activity /// cysteine-type peptidase activity /// hydrolase activity /// caspase activity
221858_at	2.052	TBC1D12	TBC1 domain family, member 12	---	regulation of Rab GTPase activity	GTPase activator activity /// Rab GTPase activator activity
1569181_x_at	2.049	---	Transcribed locus	---	---	---
236510_at	2.047	---	MRNA sequence	---	---	---
219362_at	2.038	MAK10	MAK10 homolog, amino-acid N-acetyltransferase subunit, (S. cerevisiae)	---	smooth muscle cell proliferation	protein binding /// transferase activity
228954_at	2.021	LYSMD4	LysM, putative peptidoglycan-binding, domain containing 4	---	cell wall catabolic process	---
226338_at	2.011	TMEM55A	transmembrane protein 55A	---	---	hydrolase activity
209031_at	2.003	CADM1	cell adhesion molecule 1	---	T cell mediated cytotoxicity /// apoptosis /// immune response /// cell cycle /// cell adhesion /// homophilic cell adhesion /// heterophilic cell adhesion /// multicellular organismal development /// spermatogenesis /// cell recognition /// cell recognition /// cell differentiation /// susceptibility to natural killer cell mediated cytotoxicity /// susceptibility to natural killer cell mediated cytotoxicity /// negative regulation of cell cycle /// positive regulation of natural killer cell mediated cytotoxicity /// positive regulation of natural killer cell mediated cytotoxicity /// positive regulation of cytokine secretion /// activated T cell proliferation /// detection of stimulus /// detection of stimulus	receptor binding /// receptor binding /// protein binding /// protein binding /// protein C-terminus binding /// PDZ domain binding /// protein homodimerization activity
204490_s_at	2.002	CD44	CD44 molecule (Indian blood group)	---	cell adhesion /// cell-matrix adhesion /// cell-cell adhesion	receptor activity /// binding /// protein binding /// protein binding /// collagen binding /// hyaluronic acid binding /// hyaluronic acid binding
1554019_s_at	1.995	C6orf182 /// C6orf182P	chromosome 6 open reading frame 182 pseudogene /// chromosome 6 open reading frame 182	---	---	---
236552_at	1.991	---	Transcribed locus	---	---	---

205632_s_at	1.976	PIP5K1B	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	---	phosphorylation /// phosphatidylinositol metabolic process	protein binding /// kinase activity /// phosphatidylinositol phosphate kinase activity /// 1-phosphatidylinositol-4-phosphate 5-kinase activity /// 1-phosphatidylinositol-4-phosphate 5-kinase activity /// transferase activity
226190_at	1.976	---	Homo sapiens, clone IMAGE:4294444, mRNA	---	---	---
236401_at	1.965	---	Transcribed locus, strongly similar to NP_060854.2 GTPase, IMAP family member 5 [Homo sapiens]	---	---	---
221616_s_at	1.95	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa	---	transcription /// transcription initiation /// regulation of transcription, DNA-dependent	DNA binding
206942_s_at	1.904	PMCH	pro-melanin-concentrating hormone	---	signal transduction /// neuropeptide signaling pathway /// synaptic transmission /// multicellular organismal development /// spermatogenesis /// spermatogenesis /// behavior /// feeding behavior /// cell differentiation	hormone activity /// melanin-concentrating hormone activity
225993_at	1.881	EARS2	glutamyl-tRNA synthetase 2, mitochondrial (putative)	---	translation /// tRNA aminoacylation for protein translation /// glutamyl-tRNA aminoacylation	nucleotide binding /// aminoacyl-tRNA ligase activity /// glutamate-tRNA ligase activity /// ATP binding /// ligase activity
223195_s_at	1.876	SESN2	sestrin 2	---	cell cycle arrest	---
229322_at	1.87	PPP2R5E	protein phosphatase 2, regulatory subunit B', epsilon isoform	Glycogen_Metabolism /// Wnt_signaling	signal transduction	binding /// protein binding /// protein phosphatase type 2A regulator activity /// protein phosphatase type 2A regulator activity
241972_at	1.861	LOC401588	hypothetical LOC401588	---	---	---
223469_at	1.855	PGPEP1	pyroglutamyl-peptidase I	---	proteolysis	pyroglutamyl-peptidase I activity /// peptidase activity /// cysteine type peptidase activity /// hydrolase activity
224034_at	1.851	---	Transcribed locus, weakly similar to NP_060312.1 hypothetical protein LOC55652 [Homo sapiens]	---	---	---
238526_at	1.843	RAB3IP	RAB3A interacting protein (rabin3)	---	transport /// protein transport	guanyl-nucleotide exchange factor activity /// protein binding /// protein binding
207130_at	1.769	ZMYND8	zinc finger, MYND-type containing 8	---	---	protein binding /// protein binding /// zinc ion binding /// kinase activity /// metal ion binding
1569503_at	1.754	HEATR5B	HEAT repeat containing 5B	---	---	binding
243832_at	1.751	WDR33	WD repeat domain 33	---	postreplication repair /// phosphate transport /// spermatogenesis	protein binding
244527_at	1.76	TRIO	Triple functional domain (PTPRF interacting)	---	protein amino acid phosphorylation /// transmembrane receptor protein tyrosine phosphatase signaling pathway /// regulation of Rho protein signal transduction	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// guanyl-nucleotide exchange factor activity /// guanyl-nucleotide exchange factor activity /// Rho guanyl-nucleotide exchange factor activity /// ATP binding /// kinase activity /// transferase activity
241073_at	1.726	---	Transcribed locus	---	---	---
222316_at	1.721	---	Transcribed locus	---	---	---
238908_at	1.699	CALU	Calumenin	---	---	calcium ion binding /// calcium ion binding /// calcium ion binding
211052_s_at	1.697	TBCD	tubulin folding cofactor D	---	protein folding /// beta-tubulin folding	binding /// chaperone binding
234962_at	1.674	---	---	---	---	---
235985_at	1.674	---	Transcribed locus	---	---	---
240628_at	1.656	---	Transcribed locus	---	---	---
1556492_a_at	1.655	---	Full length insert cDNA clone ZD28F11	---	---	---
214227_at	1.644	GNG7	Guanine nucleotide binding protein (G protein), gamma 7	Calcium_regulation_in_cardiac_cells /// G_Protein_Signaling /// Smooth_muscle_contraction	behavioral fear response /// signal transduction /// receptor guanylyl cyclase signaling pathway /// G-protein coupled receptor protein signaling pathway /// locomotory behavior /// regulation of G-protein coupled receptor protein signaling pathway	signal transducer activity
1558401_at	1.59	---	CDNA FLJ37332 fis, clone BRAMY2019710	---	---	---
1555881_s_at	1.585	LZTS2	leucine zipper, putative tumor suppressor 2	---	cell cycle /// negative regulation of cell cycle	---
233187_s_at	1.584	---	Clone IMAGE:113308 mRNA sequence	---	---	---
1558163_at	1.579	PEX13	Peroxisome biogenesis factor 13	---	fatty acid alpha-oxidation /// neuron migration /// suckling behavior /// transport /// locomotory behavior /// protein transport /// protein import into peroxisome matrix, docking /// protein import into peroxisome matrix, docking /// protein import into peroxisome matrix, docking /// cerebral cortex cell migration /// microtubule-based peroxisome localization	protein binding
228934_x_at	1.579	---	Transcribed locus	---	---	---
222071_s_at	1.567	SLCO4C1	solute carrier organic anion transporter family, member 4C1	---	transport	transporter activity
221957_at	1.55	PDK3	pyruvate dehydrogenase kinase, isozyme 3	Krebs-TCA_Cycle	carbohydrate metabolic process /// glucose metabolic process /// glucose metabolic process /// signal transduction /// phosphorylation /// peptidyl-histidine phosphorylation	two-component sensor activity /// protein kinase activity /// protein histidine kinase activity /// pyruvate dehydrogenase (acetyl-transferring) kinase activity /// pyruvate dehydrogenase (acetyl-transferring) kinase activity /// ATP binding /// kinase activity /// transferase activity /// transferase activity, transferring phosphorus-containing groups
227928_at	1.549	C12orf48	chromosome 12 open reading frame 48	---	---	DNA binding
240352_at	1.546	---	Transcribed locus	---	---	---
212752_at	1.544	CLASP1	cytoplasmic linker associated protein 1	---	microtubule cytoskeleton organization and biogenesis /// microtubule bundle formation /// negative regulation of microtubule depolymerization /// negative regulation of microtubule depolymerization /// cell cycle /// mitosis /// establishment and/or maintenance of cell polarity /// exit from mitosis /// negative regulation of microtubule polymerization or depolymerization /// cell division	binding /// protein binding /// microtubule binding /// microtubule binding /// kinetochore binding /// microtubule plus-end binding
203974_at	1.543	HDHD1A	haloacid dehalogenase-like hydrolase domain containing 1A	---	metabolic process	catalytic activity
202940_at	1.536	WNK1	WNK lysine deficient protein kinase 1	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// ion transport /// protein kinase cascade /// regulation of cellular process	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein kinase inhibitor activity /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity
231971_at	1.534	FANCM	Fanconi anemia, complementation group M	---	DNA metabolic process /// DNA repair /// response to DNA damage stimulus	nucleotide binding /// nucleic acid binding /// DNA binding /// helicase activity /// nuclease activity /// protein binding /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity
242089_at	1.53	---	CDNA FLJ30383 fis, clone BRACE2008102	---	---	---
230172_at	1.492	FAM14B	Family with sequence similarity 14, member B	---	---	---
227793_at	1.479	---	---	---	---	---
232693_s_at	1.442	FBXO16 /// ZNF395	zinc finger protein 395 /// F-box protein 16	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// ubiquitin cycle	nucleic acid binding /// DNA binding /// DNA binding /// zinc ion binding /// metal ion binding
1569254_s_at	1.423	INTS4	integrator complex subunit 4	---	snRNA processing	binding /// protein binding
215978_x_at	1.41	LOC152719	hypothetical protein LOC152719	---	---	---
1562416_at	1.407	---	CDNA clone IMAGE:5272221	---	---	---
1554599_x_at	1.402	---	MRNA; cDNA DKFZp547K189 (from clone DKFZp547K189)	---	---	---
200739_s_at	1.379	SUMO3	SMT3 suppressor of mif two 3 homolog 3 (S. cerevisiae)	Circadian_Exercise	protein modification process /// ubiquitin cycle	protein binding
209077_at	1.358	TXN2	thioredoxin 2	---	glycerol ether metabolic process /// transport /// cell redox homeostasis /// oxidation reduction	electron carrier activity /// protein disulfide oxidoreductase activity /// thiol-disulfide exchange intermediate activity
242984_at	1.352	MKLN1	muskelin 1, intracellular mediator containing kelch motifs	---	cell motility /// cell-matrix adhesion /// signal transduction	protein binding
239407_at	1.336	---	CDNA clone IMAGE:4837199	---	---	---
1556125_at	1.307	GPATCH2	G patch domain containing 2	---	---	nucleic acid binding
243096_at	1.285	---	Transcribed locus	---	---	---
212529_at	1.233	LSM12	LSM12 homolog (S. cerevisiae)	---	---	---
204571_x_at	1.21	PIN4	protein (peptidylprolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	---	protein folding /// protein folding	peptidyl-prolyl cis-trans isomerase activity /// isomerase activity

221673_s_at	1.199	CSNK1G1	casein kinase 1, gamma 1	---	protein amino acid phosphorylation /// Wnt receptor signaling pathway	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// ATP binding /// kinase activity /// transferase activity
226970_at	1.183	FBXO33	F-box protein 33	---	ubiquitin cycle	---
215816_at	1.182	LOC91316	similar to bK246H3.1 (immunoglobulin lambda-like polypeptide 1, pre-B-cell specific)	---	carbohydrate metabolic process	hydrolase activity, hydrolyzing O-glycosyl compounds
243705_at	1.15	DDHD1	DDHD domain containing 1	---	lipid catabolic process	hydrolase activity /// metal ion binding

GENES DOWNREGULATED EXCLUSIVELY IN NAIVE B CELLS UPON TRANSFORMATION BY EBV

Probe Set ID	Fold Change	Gene Symbol	Gene Title	Pathway	go biological process term	go molecular function term
1562453_at	-95.22	---	Transcribed locus	---	---	---
217979_at	-94.72	TSPAN13	tetraspanin 13	---	---	---
203140_at	-76.25	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	---	protein import into nucleus, translocation /// negative regulation of transcription from RNA polymerase II promoter /// negative regulation of transcription from RNA polymerase II promoter /// cell morphogenesis /// negative regulation of cell-matrix adhesion /// germinal center formation /// regulation of germinal center formation /// negative regulation of T-helper 2 type immune response /// negative regulation of B cell apoptosis /// transcription /// regulation of transcription, DNA-dependent /// response to DNA damage stimulus /// Rho protein signal transduction /// spermatogenesis /// protein localization /// negative regulation of cell proliferation /// actin cytoskeleton organization and biogenesis /// B cell differentiation /// negative regulation of cell growth /// positive regulation of B cell proliferation /// regulation of Rho GTPase activity /// negative regulation of mast cell cytokine production /// negative regulation of Rho protein signal transduction /// T-helper 2 type immune response /// positive regulation of apoptosis /// negative regulation of apoptosis /// regulation of memory T cell differentia	nucleic acid binding /// DNA binding /// chromatin binding /// chromatin binding /// protein binding /// protein binding /// zinc ion binding /// transcription repressor activity /// transcription repressor activity /// transcription repressor activity /// sequence-specific DNA binding /// sequence-specific DNA binding /// metal ion binding
218807_at	-54.91	VAV3	vav 3 guanine nucleotide exchange factor	Integrin-mediated_cell_adhesion_KEGG	intracellular signaling cascade /// small GTPase mediated signa transduction /// regulation of Rho protein signal transduction	SH3/SH2 adaptor activity /// guanyl-nucleotide exchange factor activity /// Rho guanyl-nucleotide exchange factor activity /// GTPase activator activity /// protein binding /// zinc ion binding /// diacylglycerol binding /// metal ion binding
224192_at	-31.49	FCRL2	Fc receptor-like 2	---	cell-cell signaling	receptor activity /// SH3/SH2 adaptor activity
228153_at	-31.44	RNF144B	ring finger 144B	---	ubiquitin cycle /// apoptosis /// protein ubiquitination during ubiquitin-dependent protein catabolic process	ubiquitin-protein ligase activity /// protein binding /// protein binding /// zinc ion binding /// electron carrier activity /// ligase activity /// metal ion binding /// iron-sulfur cluster binding
236796_at	-29.02	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// protein binding /// sequence-specific DNA binding /// protein dimerization activity
230983_at	-28.27	FAM129C	family with sequence similarity 129, member C	---	---	---
229723_at	-27.33	TAGAP	T-cell activation RhoGTPase activating protein	---	signal transduction	guanyl-nucleotide exchange factor activity
1565034_s_at	-26.91	AFF3 /// MLL	AF4/FMR2 family, member 3 /// myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	---	DNA repair /// DNA recombination /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// protein complex assembly /// protein amino acid phosphorylation /// apoptosis /// cell cycle /// multicellular organismal development /// embryonic hemopoiesis	nucleic acid binding /// DNA binding /// transcription factor activity /// RNA polymerase II transcription factor activity /// protein kinase activity /// protein binding /// protein binding /// ATP binding /// GTP binding /// zinc ion binding /// identical protein binding /// protein homodimerization activity /// metal ion binding
1555745_a_at	-26.77	LYZ	lysozyme (renal amyloidosis)	---	inflammatory response /// metabolic process /// cell wall catabolic process /// cytolysis /// defense response to bacterium	lysozyme activity /// lysozyme activity /// catalytic activity /// protein binding /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds
228377_at	-25.34	KLHL14	kelch-like 14 (Drosophila)	---	---	protein binding
219304_s_at	-25.33	PDGFD	platelet derived growth factor D	---	cell proliferation	growth factor activity
219799_s_at	-24.1	DHRS9	dehydrogenase/reductase (SDR family) member 9	---	metabolic process /// androgen metabolic process /// androgen metabolic process /// epithelial cell differentiation /// progesterone metabolic process /// retinol metabolic process /// 9-cis-retinoic acid biosynthetic process /// 9-cis-retinoic acid biosynthetic process	catalytic activity /// alcohol dehydrogenase activity /// alcohol dehydrogenase activity /// retinol dehydrogenase activity /// retinol dehydrogenase activity /// binding /// oxidoreductase activity /// racemase and epimerase activity /// 3-alpha(17-beta)-hydroxysteroid dehydrogenase (NAD) activity /// 3-alpha(17-beta)-hydroxysteroid dehydrogenase (NAD) activity
1552541_at	-23.16	TAGAP	T-cell activation RhoGTPase activating protein	---	signal transduction	guanyl-nucleotide exchange factor activity
226818_at	-22.92	MPEG1	macrophage expressed gene 1	---	---	---
226841_at	-22.82	MPEG1	macrophage expressed gene 1	---	---	---
236735_at	-21.98	---	Full length insert cDNA clone ZC64D04	---	---	---
1552542_s_at	-21.4	TAGAP	T-cell activation RhoGTPase activating protein	---	signal transduction	guanyl-nucleotide exchange factor activity
227198_at	-21.21	AFF3	AF4/FMR2 family, member 3	---	transcription /// regulation of transcription, DNA-dependent /// multicellular organismal development	DNA binding
235841_at	-21.13	---	Transcribed locus	---	---	---
228167_at	-19.87	KLHL6	kelch-like 6 (Drosophila)	---	---	protein binding
235000_at	-19.83	---	CDNA FLJ30652 fis, clone DFNES2000011	---	---	---
1560397_s_at	-19.35	KLHL6	kelch-like 6 (Drosophila)	---	---	protein binding
232286_at	-18.55	---	CDNA FLJ12187 fis, clone MAMMA1000831	---	---	---
218692_at	-18.29	GOLSYN	Golgi-localized protein	---	---	---
233111_at	-18.27	---	CDNA FLJ13886 fis, clone THYRO1001559	---	---	---
205916_at	-18.04	S100A7	S100 calcium binding protein A7	---	response to reactive oxygen species /// angiogenesis /// epidermis development /// keratinocyte differentiation /// innate immune response /// defense response to Gram-negative bacterium /// sequestering of metal ion	calcium ion binding /// calcium ion binding /// protein binding /// protein binding /// zinc ion binding /// zinc ion binding /// zinc ion binding /// metal ion binding
238769_at	-17.38	---	Transcribed locus	---	---	---
1558662_s_at	-17.03	BANK1	B-cell scaffold protein with ankyrin repeats 1	---	B cell activation	---
201008_s_at	-15.64	TXNIP	thioredoxin interacting protein	---	transcription /// regulation of transcription, DNA-dependent /// cell cycle /// keratinocyte differentiation	protein binding
1558732_at	-14.13	MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// response to stress /// protein kinase cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// small GTPase regulator activity /// ATP binding /// ATP binding /// kinase activity /// transferase activity
226017_at	-13.88	CMTM7	CKLF-like MARVEL transmembrane domain containing 7	---	chemotaxis	cytokine activity
242899_at	-13.17	---	CDNA FLJ33813 fis, clone CTONG2002744	---	---	---
222915_s_at	-12.7	BANK1	B-cell scaffold protein with ankyrin repeats 1	---	B cell activation	---
229064_s_at	-12.55	RCAN3	RCAN family member 3	---	anatomical structure morphogenesis /// calcium-mediated signaling	RNA binding /// troponin I binding
1558826_at	-12.54	ZNF831	zinc finger protein 831	---	---	nucleic acid binding /// zinc ion binding /// metal ion binding
236302_at	-12.43	PPM1E	protein phosphatase 1E (PP2C domain containing)	---	protein amino acid dephosphorylation	magnesium ion binding /// catalytic activity /// phosphoprotein phosphatase activity /// protein serine/threonine phosphatase activity /// hydrolase activity /// manganese ion binding /// metal ion binding
224928_at	-12.37	SETD7	SET domain containing (lysine methyltransferase) 7	---	transcription /// regulation of transcription, DNA-dependent /// chromatin modification /// chromatin modification	protein binding /// methyltransferase activity /// transferase activity /// histone-lysine N-methyltransferase activity /// histone-lysine N-methyltransferase activity
201010_s_at	-12.06	TXNIP	thioredoxin interacting protein	---	transcription /// regulation of transcription, DNA-dependent /// cell cycle /// keratinocyte differentiation	protein binding
217809_at	-11.76	BZW2	basic leucine zipper and W2 domains 2	---	regulation of translational initiation /// multicellular organismal development /// nervous system development /// cell differentiation	translation initiation factor activity /// binding /// protein binding /// protein binding
235150_at	-11.6	---	CDNA FLJ33813 fis, clone CTONG2002744	---	---	---
213906_at	-11.13	MYBL1	v-myb myeloblastosis viral oncogene homolog (avian)-like 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription activator activity /// transcription regulator activity
224735_at	-11.05	CYBASC3	cytochrome b, ascorbate dependent 3	---	transport /// oxidation reduction	iron ion binding /// oxidoreductase activity /// metal ion binding
226272_at	-10.58	RCAN3	RCAN family member 3	---	anatomical structure morphogenesis /// calcium-mediated signaling	RNA binding /// troponin I binding
226122_at	-10.19	PLEKHG1	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	---	regulation of Rho protein signal transduction	guanyl-nucleotide exchange factor activity /// Rho guanyl-nucleotide exchange factor activity
207001_x_at	-9.941	TSC22D3	TSC22 domain family, member 3	---	regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	transcription factor activity /// transcription factor activity

213624_at	-9.826	SMPDL3A	sphingomyelin phosphodiesterase, acid-like 3A	---	metabolic process	protein binding /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds
206571_s_at	-9.64	MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// response to stress /// protein kinase cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// small GTPase regulator activity /// ATP binding /// ATP binding /// kinase activity /// transferase activity
212458_at	-8.999	SPRED2	sprouty-related, EVH1 domain containing 2	---	inactivation of MAPK activity /// inactivation of MAPK activity /// multicellular organismal development /// regulation of signal transduction	stem cell factor receptor binding /// stem cell factor receptor binding /// protein binding /// protein binding
228175_at	-8.974	---	CDNA FLJ31660 fis, clone NT2RI2004410	---	---	---
205174_s_at	-8.755	QPCT	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	---	protein modification process /// proteolysis	peptidase activity /// zinc ion binding /// acyltransferase activity /// glutaminyl-peptide cyclotransferase activity /// transferase activity /// metal ion binding
1565838_at	-8.647	---	CDNA FLJ35990 fis, clone TESTI2014415	---	---	---
235102_x_at	-8.647	---	Transcribed locus	---	---	---
244097_at	-8.61	CR2	complement component (3d/Epstein Barr virus) receptor 2	---	immune response /// immune response /// complement activation, classical pathway /// innate immune response	receptor activity /// complement receptor activity /// complement receptor activity /// transmembrane receptor activity /// protein homodimerization activity
241577_at	-8.361	---	---	---	---	---
1562269_at	-8.279	---	MRNA; cDNA DKFZp686G1636 (from clone DKFZp686G1636)	---	---	---
244770_at	-8.135	---	Transcribed locus	---	---	---
204446_s_at	-8.08	ALOX5	arachidonate 5-lipoxygenase	Eicosanoid_Synthesis	leukotriene metabolic process /// leukotriene metabolic process /// inflammatory response /// inflammatory response /// leukotriene biosynthetic process /// oxidation reduction	arachidonate 5-lipoxygenase activity /// arachidonate 5-lipoxygenase activity /// iron ion binding /// calcium ion binding /// protein binding /// lipoxygenase activity /// oxidoreductase activity /// oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen /// metal ion binding
1564164_at	-7.949	C1orf218	chromosome 1 open reading frame 218	---	---	---
227268_at	-7.859	RNFT1	ring finger protein, transmembrane 1	---	---	protein binding /// zinc ion binding /// metal ion binding
235349_at	-7.786	FAM82A	family with sequence similarity 82, member A	---	---	binding
244781_x_at	-7.675	---	Transcribed locus	---	---	---
207522_s_at	-7.496	ATP2A3	ATPase, Ca transporting, ubiquitous	Calcium_regulation_in_cardiac_cells /// Smooth_muscle_contraction	transport /// transport /// ion transport /// cation transport /// calcium ion transport /// calcium ion transport /// metabolic process /// proton transport	nucleotide binding /// magnesium ion binding /// catalytic activity /// calcium-transporting ATPase activity /// calcium-transporting ATPase activity /// calcium ion binding /// protein binding /// ATP binding /// ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism /// hydrolase activity /// hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances /// metal ion binding
210796_x_at	-7.435	SIGLEC6	sialic acid binding Ig-like lectin 6	---	cell adhesion /// cell-cell signaling	protein binding /// sugar binding
212538_at	-7.334	DOCK9	dedicator of cytokinesis 9	---	---	guanyl-nucleotide exchange factor activity /// binding /// GTP binding /// GTPase binding
1556579_s_at	-7.307	IGSF10	immunoglobulin superfamily, member 10	---	ossification /// protein amino acid phosphorylation /// multicellular organismal development /// cell differentiation	vascular endothelial growth factor receptor activity /// protein binding /// ATP binding
226344_at	-7.252	ZMAT1	zinc finger, matrin type 1	---	---	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
208820_at	-7.25	PTK2	PTK2 protein tyrosine kinase 2	Integrin-mediated_cell_adhesion_KEGG	protein amino acid phosphorylation /// protein amino acid phosphorylation /// signal complex assembly /// integrin-mediated signaling pathway	nucleotide binding /// protein kinase activity /// protein tyrosine kinase activity /// protein tyrosine kinase activity /// non-membrane spanning protein tyrosine kinase activity /// signal transducer activity /// protein binding /// ATP binding /// kinase activity /// transferase activity /// SH2 domain binding
1560754_at	-7.244	CMTM7	CKLF-like MARVEL transmembrane domain containing 7	---	chemotaxis	cytokine activity
232303_at	-7.212	ZNF608	zinc finger protein 608	---	---	nucleic acid binding /// zinc ion binding /// metal ion binding
213036_x_at	-7.061	ATP2A3	ATPase, Ca transporting, ubiquitous	Calcium_regulation_in_cardiac_cells /// Smooth_muscle_contraction	transport /// transport /// ion transport /// cation transport /// calcium ion transport /// calcium ion transport /// metabolic process /// proton transport	nucleotide binding /// magnesium ion binding /// catalytic activity /// calcium-transporting ATPase activity /// calcium-transporting ATPase activity /// calcium ion binding /// protein binding /// ATP binding /// ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism /// hydrolase activity /// hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances /// metal ion binding
1561872_at	-6.996	---	---	---	---	---
1559812_at	-6.992	---	CDNA clone IMAGE:5277868	---	---	---
229383_at	-6.646	---	CDNA FLJ34016 fis, clone FCBBF2002541	---	---	---
206398_s_at	-6.633	CD19	CD19 molecule	---	cellular defense response /// cell surface receptor linked signal transduction	receptor signaling protein activity /// protein binding
230777_s_at	-6.408	PRDM15	PR domain containing 15	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// DNA binding /// zinc ion binding /// metal ion binding
238951_at	-6.229	---	Transcribed locus	---	---	---
235251_at	-6.226	---	CDNA FLJ42786 fis, clone BRAWH3006761	---	---	---
202016_at	-6.218	MEST	mesoderm specific transcript homolog (mouse)	---	mesoderm development	catalytic activity /// protein binding
203510_at	-6.001	MET	met proto-oncogene (hepatocyte growth factor receptor)	---	activation of MAPK activity /// neuron migration /// protein amino acid phosphorylation /// signal transduction /// cell surface receptor linked signal transduction /// multicellular organismal development /// brain development /// muscle development /// lactation /// cell proliferation /// sperm motility /// adult behavior /// protein amino acid autophosphorylation /// hepatocyte growth factor receptor signaling pathway /// myoblast proliferation	nucleotide binding /// protein kinase activity /// protein tyrosine kinase activity /// protein tyrosine kinase activity /// transmembrane receptor protein tyrosine kinase activity /// receptor activity /// hepatocyte growth factor receptor activity /// hepatocyte growth factor receptor activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity
219498_s_at	-5.954	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	---	transcription /// regulation of transcription, DNA-dependent /// hemopoiesis	nucleic acid binding /// zinc ion binding /// metal ion binding
1554140_at	-5.931	WDR78	WD repeat domain 78	---	---	---
232874_at	-5.908	DOCK9	Dedicator of cytokinesis 9	---	---	guanyl-nucleotide exchange factor activity /// GTP binding /// GTPase binding
204192_at	-5.878	CD37	CD37 molecule	---	protein amino acid N-linked glycosylation	---
214367_at	-5.767	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	---	intracellular signaling cascade /// small GTPase mediated signal transduction /// regulation of small GTPase mediated signal transduction	guanyl-nucleotide exchange factor activity /// calcium ion binding /// zinc ion binding /// diacylglycerol binding /// metal ion binding
239205_s_at	-5.703	CR1 /// CR1L	complement component (3b/4b) receptor 1 (Knops blood group) /// complement component (3b/4b) receptor 1-like	---	immune response /// complement activation /// complement activation, classical pathway /// innate immune response	receptor activity /// complement receptor activity /// complement component C3b receptor activity
208488_s_at	-5.587	CR1	complement component (3b/4b) receptor 1 (Knops blood group)	---	immune response /// complement activation /// complement activation, classical pathway /// innate immune response	receptor activity /// complement receptor activity /// complement component C3b receptor activity
36920_at	-5.569	MTM1	myotubularin 1	---	protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// muscle development /// dephosphorylation /// phospholipid dephosphorylation	inositol or phosphatidylinositol phosphatase activity /// phosphoprotein phosphatase activity /// protein serine/threonine phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// hydrolase activity /// phosphoric monoester hydrolase activity
230803_s_at	-5.556	ARHGAP24	Rho GTPase activating protein 24	---	angiogenesis /// signal transduction /// multicellular organismal development /// cell differentiation	GTPase activator activity /// protein binding
1568604_a_at	-5.538	CADPS	Ca2+-dependent secretion activator	---	transport /// exocytosis /// exocytosis /// protein transport	calcium ion binding /// lipid binding /// metal ion binding
211181_x_at	-5.526	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	---	generation of precursor metabolites and energy /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// multicellular organismal development /// multicellular organismal development /// positive regulation of granulocyte differentiation /// regulation of transcription /// positive regulation of angiogenesis /// positive regulation of transcription from RNA polymerase II promoter	nucleic acid binding /// DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// protein binding /// ATP binding /// ATP binding /// zinc ion binding /// transcription activator activity /// chloride ion binding /// metal ion binding

200666_s_at	-5.512	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	---	protein folding /// response to stress /// response to unfolded protein /// chaperone cofactor-dependent protein folding	heat shock protein binding /// unfolded protein binding
244313_at	-5.412	CR1	complement component (3b/4b) receptor 1 (Knops blood group)	---	immune response /// complement activation /// complement activation, classical pathway /// innate immune response	receptor activity /// complement receptor activity /// complement component C3b receptor activity
1555996_s_at	-5.354	EIF4A2	eukaryotic translation initiation factor 4A, isoform 2	---	translation /// regulation of translational initiation	nucleotide binding /// nucleic acid binding /// RNA binding /// translation initiation factor activity /// translation initiation factor activity /// protein binding /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity
244263_at	-5.276	---	---	---	---	---
205301_s_at	-5.2	OGG1	8-oxoguanine DNA glycosylase	---	DNA repair /// DNA repair /// base-excision repair /// base-excision repair /// nucleotide-excision repair /// response to DNA damage stimulus /// metabolic process	DNA binding /// damaged DNA binding /// damaged DNA binding /// catalytic activity /// DNA-(apurinic or apyrimidinic site) lyase activity /// endonuclease activity /// protein binding /// oxidized purine base lesion DNA N-glycosylase activity /// oxidized purine base lesion DNA N-glycosylase activity /// oxidized purine base lesion DNA N-glycosylase activity /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds /// lyase activity
232461_at	-5.166	AHI1	Abelson helper integration site 1	---	---	---
209717_at	-5.066	EVI5	ecotropic viral integration site 5	---	cell cycle /// multicellular organismal development /// cell proliferation /// regulation of Rab GTPase activity /// cell division	Rab GTPase activator activity /// protein binding /// protein binding
244669_at	-5.028	SNHG5 /// SNORD50A /// SNORD50B	small nucleolar RNA, C/D box 50A /// small nucleolar RNA host gene (non-protein coding) 5 /// small nucleolar RNA, C/D box 50B	---	---	---
226246_at	-4.941	STIM2	stromal interaction molecule 2	---	transport /// ion transport /// calcium ion transport /// negative regulation of calcium ion transport via store-operated calcium channel	calcium ion binding /// protein binding
223707_at	-4.915	RPL27A	ribosomal protein L27a	Ribosomal_Proteins	translation /// translation	RNA binding /// structural constituent of ribosome /// structural constituent of ribosome
241462_at	-4.871	---	Transcribed locus, strongly similar to XP_001139387.1 PREDICTED: hypothetical protein [Pan troglodytes]	---	---	---
205039_s_at	-4.866	IKZF1	IKAROS family zinc finger 1 (Ikaros)	---	transcription /// regulation of transcription, DNA-dependent /// mesoderm development	nucleic acid binding /// DNA binding /// DNA binding /// zinc ion binding /// metal ion binding
220206_at	-4.823	ZMYM1	zinc finger, MYM-type 1	---	---	zinc ion binding /// metal ion binding /// protein dimerization activity
219681_s_at	-4.75	RAB11FIP1	RAB11 family interacting protein 1 (class I)	---	transport /// protein transport	protein binding
228987_at	-4.717	---	Transcribed locus	---	---	---
1553088_a_at	-4.711	BCL2L11	BCL2-like 11 (apoptosis facilitator)	Apoptosis	apoptosis /// induction of apoptosis /// positive regulation of apoptosis	protein binding
229253_at	-4.704	THEM4	thioesterase superfamily member 4	---	---	---
226912_at	-4.657	ZDHHC23	zinc finger, DHHC-type containing 23	---	---	zinc ion binding /// acyltransferase activity /// transferase activity /// metal ion binding
232739_at	-4.635	SPIB	Spi-B transcription factor (Spi-1/PU.1 related)	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// macrophage differentiation	DNA binding /// transcription factor activity /// RNA polymerase II transcription factor activity /// sequence-specific DNA binding
226878_at	-4.629	HLA-DOA	major histocompatibility complex, class II, DO alpha	---	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II /// immune response /// antigen processing and presentation	MHC class II receptor activity
1554161_at	-4.573	SLC25A27	solute carrier family 25, member 27	Electron_Transport_Chain	generation of precursor metabolites and energy /// transport /// mitochondrial transport	transporter activity /// binding
240039_at	-4.572	PLA2R1	phospholipase A2 receptor 1, 180kDa	---	endocytosis	receptor activity /// receptor activity /// binding /// sugar binding
204101_at	-4.544	MTM1	myotubularin 1	---	protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// muscle development /// dephosphorylation /// phospholipid dephosphorylation	inositol or phosphatidylinositol phosphatase activity /// phosphoprotein phosphatase activity /// protein serine/threonine phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// hydrolase activity /// phosphoric monoester hydrolase activity
232112_at	-4.476	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	---	small GTPase mediated signal transduction	guanyl-nucleotide exchange factor activity
229393_at	-4.447	L3MBTL3	l(3)mbt-like 3 (Drosophila)	---	regulation of transcription	---
239292_at	-4.392	---	Transcribed locus	---	---	---
235080_at	-4.263	---	Transcribed locus	---	---	---
241534_at	-4.239	---	Homo sapiens, clone IMAGE:4698842, mRNA	---	---	---
213521_at	-4.237	PTPN18	protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	---	protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// dephosphorylation	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// non-membrane spanning protein tyrosine phosphatase activity /// receptor activity /// hydrolase activity /// phosphoric monoester hydrolase activity
225997_at	-4.182	MOBKL1A	MOB1, Mps One Binder kinase activator-like 1A (yeast)	---	protein amino acid autophosphorylation	protein binding /// zinc ion binding /// kinase activator activity /// kinase binding /// metal ion binding
202723_s_at	-4.164	FOXO1	forkhead box O1	---	blood vessel development /// transcription /// regulation of transcription, DNA-dependent /// anti-apoptosis /// insulin receptor signaling pathway /// regulation of cell proliferation /// positive regulation of transcription from RNA polymerase II promoter	DNA binding /// transcription factor activity /// protein binding /// protein binding /// transcription activator activity /// sequence-specific DNA binding /// sequence-specific DNA binding
232262_at	-4.158	PIGL	phosphatidylinositol glycan anchor biosynthesis, class L	---	GPI anchor biosynthetic process /// GPI anchor biosynthetic process	N-acetylglucosaminylphosphatidylinositol deacetylase activity /// N-acetylglucosaminylphosphatidylinositol deacetylase activity /// hydrolase activity
209829_at	-4.157	C6orf32	chromosome 6 open reading frame 32	---	multicellular organismal development /// skeletal muscle development /// cell differentiation	binding
1554306_at	-4.121	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B	---	signal transduction	nucleotide binding /// calmodulin binding /// ATP binding /// inositol trisphosphate 3-kinase activity /// inositol trisphosphate kinase activity /// kinase activity /// transferase activity
238917_s_at	-4.113	MGC24039	hypothetical protein MGC24039	---	---	---
1554481_a_at	-4.107	EPB41	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	---	blood circulation /// actin cytoskeleton organization and biogenesis /// cortical actin cytoskeleton organization and biogenesis	actin binding /// structural molecule activity /// structural constituent of cytoskeleton /// binding /// protein binding /// phosphatidylinositol binding /// cytoskeletal protein binding
226124_at	-4.004	PPP1R9B	protein phosphatase 1, regulatory (inhibitor) subunit 9B	---	regulation of cell growth by extracellular stimulus /// cell cycle arrest /// regulation of exit from mitosis /// multicellular organismal development /// nervous system development /// RNA splicing /// cell differentiation /// negative regulation of cell growth /// regulation of cell proliferation	actin binding /// protein phosphatase inhibitor activity /// protein binding /// protein binding /// protein phosphatase 1 binding
1555372_at	-3.986	BCL2L11	BCL2-like 11 (apoptosis facilitator)	Apoptosis	apoptosis /// induction of apoptosis /// positive regulation of apoptosis	protein binding
244653_at	-3.971	SETD7	SET domain containing (lysine methyltransferase) 7	---	transcription /// regulation of transcription, DNA-dependent /// chromatin modification /// chromatin modification	protein binding /// methyltransferase activity /// transferase activity /// histone-lysine N-methyltransferase activity /// histone-lysine N-methyltransferase activity
241267_at	-3.948	EHD3	EH-domain containing 3	---	---	nucleotide binding /// nucleic acid binding /// GTPase activity /// calcium ion binding /// ATP binding /// GTP binding
215249_at	-3.904	RPL36A	ribosomal protein L36a	Ribosomal_Proteins	translation	tRNA binding /// RNA binding /// structural constituent of ribosome /// protein binding /// protein binding
1554692_at	-3.9	SLC23A2	solute carrier family 23 (nucleobase transporters), member 2	---	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process /// transport /// ion transport /// sodium ion transport /// nucleobase transport /// molecular hydrogen transport /// L-ascorbic acid metabolic process	transporter activity /// sodium-dependent multivitamin transmembrane transporter activity /// nucleobase transmembrane transporter activity /// symporter activity /// sodium ion binding
231323_at	-3.884	PSMB2	proteasome (prosome, macropain) subunit, beta type, 2	Proteasome_Degradation	ubiquitin-dependent protein catabolic process	threonine endopeptidase activity /// peptidase activity /// hydrolase activity
1559902_at	-3.864	MKL1	megakaryoblastic leukemia (translocation) 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	nucleotide binding /// nucleic acid binding /// RNA binding /// binding /// protein binding
227364_at	-3.862	PAG1	phosphoprotein associated with glycosphingolipid microdomains 1	---	immune response /// signal transduction /// intracellular signaling cascade /// regulation of T cell activation /// negative regulation of T cell activation	SH3/SH2 adaptor activity /// protein binding /// protein binding /// SH2 domain binding
1555209_at	-3.86	LOC439951	hypothetical LOC439951	---	---	---

206370_at	-3.859	PIK3CG	phosphoinositide-3-kinase, catalytic, gamma polypeptide	---	protein amino acid phosphorylation /// G-protein coupled receptor protein signaling pathway /// negative regulation of apoptosis /// phosphoinositide phosphorylation /// phosphoinositide-mediated signaling	inositol or phosphatidylinositol kinase activity /// binding /// protein binding /// protein binding /// kinase activity /// 1-phosphatidylinositol-3-kinase activity /// 1-phosphatidylinositol-3-kinase activity /// transferase activity /// phosphotransferase activity, alcohol group as acceptor /// phosphatidylinositol-4,5-bisphosphate 3-kinase activity
232945_at	-3.853	PUS10	Pseudouridylate synthase 10	---	tRNA processing	isomerase activity
212856_at	-3.85	GRAMD4	GRAM domain containing 4	---	apoptosis	---
1569608_x_at	-3.764	LOC643187	hypothetical LOC643187	---	---	---
207164_s_at	-3.751	ZNF238	zinc finger protein 238	---	negative regulation of transcription from RNA polymerase II promoter /// transcription /// regulation of transcription, DNA-dependent /// transport	nucleic acid binding /// DNA binding /// DNA binding /// transcription factor activity /// specific RNA polymerase II transcription factor activity /// protein binding /// protein binding /// zinc ion binding /// metal ion binding
218032_at	-3.729	SNN	stannin	---	response to stress /// response to abiotic stimulus	---
207238_s_at	-3.708	PTPRC	protein tyrosine phosphatase, receptor type, C	---	negative regulation of T cell mediated cytotoxicity /// negative regulation of cytokine and chemokine mediated signaling pathway /// immunoglobulin biosynthetic process /// negative regulation of protein kinase activity /// negative regulation of protein kinase activity /// protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// cell surface receptor linked signal transduction /// dephosphorylation /// dephosphorylation /// T cell differentiation /// positive regulation of B cell proliferation /// regulation of S phase /// B cell proliferation /// positive regulation of T cell proliferation /// positive regulation of protein kinase activity /// T cell receptor signaling pathway /// B cell receptor signaling pathway /// positive regulation of antigen receptor-mediated signaling pathway /// release of sequestered calcium ion into cytosol /// defense response to virus /// regulation of cell cycle	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// receptor activity /// transmembrane receptor protein tyrosine phosphatase activity /// protein binding /// hydrolase activity /// phosphoric monoester hydrolase activity /// protein kinase binding
210435_at	-3.684	ARL17 /// ARL17P1	ADP-ribosylation factor-like 17 pseudogene 1 /// ADP-ribosylation factor-like 17	---	transport /// protein transport /// vesicle-mediated transport	nucleotide binding /// GTP binding
203144_s_at	-3.682	KIAA0040	KIAA0040	---	---	---
219481_at	-3.68	TTC13	tetratricopeptide repeat domain 13	---	---	binding
235400_at	-3.672	FCRLA	Fc receptor-like A	---	cell differentiation	receptor activity
206527_at	-3.664	ABAT	4-aminobutyrate aminotransferase	---	behavior /// gamma-aminobutyric acid metabolic process /// gamma-aminobutyric acid catabolic process /// neurotransmitter catabolic process /// neurotransmitter catabolic process /// behavioral response to cocaine	catalytic activity /// 4-aminobutyrate transaminase activity /// 4-aminobutyrate transaminase activity /// transaminase activity /// transferase activity /// pyridoxal phosphate binding /// pyridoxal phosphate binding /// succinate-semialdehyde dehydrogenase binding /// protein homodimerization activity /// (S)-3-amino-2-methylpropionate transaminase activity
231773_at	-3.582	ANGPTL1	angiopoietin-like 1	---	signal transduction	receptor binding /// receptor binding
240007_at	-3.56	---	Transcribed locus	---	---	---
211468_s_at	-3.524	RECQL5	RecQ protein-like 5	---	DNA metabolic process /// DNA repair /// DNA recombination	nucleotide binding /// nucleic acid binding /// DNA helicase activity /// DNA helicase activity /// helicase activity /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity
243307_at	-3.516	---	Transcribed locus	---	---	---
235179_at	-3.504	ZNF641	zinc finger protein 641	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
225024_at	-3.48	C20orf77	chromosome 20 open reading frame 77	---	---	---
235385_at	-3.455	---	CDNA FLJ34016 fis, clone FCBBF2002541	---	---	---
214330_at	-3.452	ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2	---	proton-transporting ATP synthase complex assembly	protein binding
1557558_s_at	-3.447	LOC100129196	Hypothetical protein LOC100131664	---	---	---
243040_at	-3.413	---	---	---	---	---
213178_s_at	-3.396	MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3	---	vesicle-mediated transport /// regulation of JNK cascade	MAP-kinase scaffold activity /// kinase activity /// kinesin binding /// protein kinase binding
243780_at	-3.387	---	CDNA FLJ46553 fis, clone THYMU3038879	---	---	---
212208_at	-3.383	MED13L	mediator complex subunit 13-like	---	transcription /// regulation of transcription, DNA-dependent	---
225423_x_at	-3.382	LOC100129015	hypothetical protein LOC100129015	---	---	---
234974_at	-3.367	GALM	Galactose mutarotase (aldose 1-epimerase)	---	carbohydrate metabolic process	catalytic activity /// aldose 1-epimerase activity /// isomerase activity /// carbohydrate binding
225172_at	-3.362	CRAMP1L /// HN1L	Crm, cramped-like (Drosophila) /// hematological and neurological expressed 1-like	---	---	DNA binding
210934_at	-3.339	BLK	B lymphoid tyrosine kinase	---	protein amino acid phosphorylation /// protein kinase cascade	nucleotide binding /// protein kinase activity /// protein tyrosine kinase activity /// protein tyrosine kinase activity /// non-membrane spanning protein tyrosine kinase activity /// protein binding /// ATP binding /// kinase activity /// transferase activity
215527_at	-3.327	KHDRBS2	KH domain containing, RNA binding, signal transduction associated 2	---	transcription /// regulation of transcription, DNA-dependent	RNA binding
1570076_at	-3.309	---	CDNA clone IMAGE:3848516	---	---	---
226509_at	-3.294	ZNF641	zinc finger protein 641	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
229814_at	-3.29	---	Transcribed locus	---	---	---
222336_at	-3.212	C4orf34	chromosome 4 open reading frame 34	---	---	protein binding
238389_s_at	-3.21	---	Transcribed locus, moderately similar to XP_001093747.1 PREDICTED: tumor protein p53 binding protein, 2 [Macaca mulatta]	---	---	---
225886_at	-3.197	---	MRNA; cDNA DKFZp434E109 (from clone DKFZp434E109)	---	---	---
232708_at	-3.196	GALT	galactose-1-phosphate uridylyltransferase	GPCRDB_Class_A_Rhodopsin-like /// Peptide_GPCRs	carbohydrate metabolic process /// galactose metabolic process /// galactose metabolic process	catalytic activity /// iron ion binding /// UDP-glucose:hexose-1-phosphate uridylyltransferase activity /// zinc ion binding /// transferase activity /// nucleotidyltransferase activity /// UTP:galactose-1-phosphate uridylyltransferase activity /// metal ion binding
211593_s_at	-3.147	MAST2	microtubule associated serine/threonine kinase 2	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// regulation of interleukin-12 biosynthetic process /// spermatid differentiation	nucleotide binding /// magnesium ion binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein binding /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity /// phosphatase binding /// metal ion binding
241053_at	-3.131	LOC730184	hypothetical LOC730184	---	---	---
227741_at	-3.127	PTPLB	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	---	---	protein binding
241328_at	-3.126	ZMAT1	zinc finger, matrin type 1	---	---	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
234215_at	-3.118	---	CDNA: FLJ21496 fis, clone COL04279	---	---	---
225269_s_at	-3.101	C2orf12	chromosome 2 open reading frame 12	---	---	---
1562352_at	-3.1	---	Homo sapiens, clone IMAGE:5760997, mRNA	---	---	---
1553936_a_at	-3.092	MGC2848	hypothetical protein MGC2848	---	---	---
239068_at	-3.092	GNL1	guanine nucleotide binding protein-like 1	---	T cell mediated immunity /// response to DNA damage stimulus /// signal transduction	nucleotide binding /// structural molecule activity /// GTP binding /// GTP binding
1554910_at	-3.064	PRKD3	protein kinase D3	G_Protein_Signaling	protein amino acid phosphorylation /// protein amino acid phosphorylation /// activation of protein kinase C activity /// intracellular signaling cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein kinase C activity /// protein kinase C activity /// protein binding /// ATP binding /// zinc ion binding /// kinase activity /// transferase activity /// diacylglycerol binding /// metal ion binding
225680_at	-3.054	LRWD1	leucine-rich repeats and WD repeat domain containing 1	---	---	protein binding
225061_at	-3.035	DNAJA4	DnaJ (Hsp40) homolog, subfamily A, member 4	---	protein folding	zinc ion binding /// heat shock protein binding /// metal ion binding /// unfolded protein binding

1554602_at	-3.012	RBM8A	RNA binding motif protein 8A	---	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay /// RNA processing /// mRNA processing /// transport /// RNA splicing /// mRNA transport	nucleotide binding /// nucleic acid binding /// RNA binding /// RNA binding /// mRNA binding /// protein binding
201750_s_at	-3.01	ECE1	endothelin converting enzyme 1	---	proteolysis /// cell-cell signaling	metalloendopeptidase activity /// neprilysin activity /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// endothelin-converting enzyme 1 activity /// hydrolase activity /// metal ion binding
243961_at	-3.009	---	Homo sapiens, clone IMAGE:4862110, mRNA	---	---	---
217745_s_at	-2.994	NAT13	N-acetyltransferase 13	---	N-terminal protein amino acid acetylation /// metabolic process	protein binding /// N-acetyltransferase activity /// acyltransferase activity /// transferase activity
243386_at	-2.992	CASZ1	castor zinc finger 1	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
227833_s_at	-2.991	MBD6	methyl-CpG binding domain protein 6	---	---	DNA binding /// structural constituent of cell wall
230339_at	-2.988	CCDC138	Coiled-coil domain containing 138	---	---	---
225081_s_at	-2.964	CDCA7L	cell division cycle associated 7-like	---	transcription /// regulation of transcription, DNA-dependent /// cell division	---
228493_at	-2.949	NKAP	NFKB activating protein	---	---	---
231708_at	-2.923	C2CD3	C2 calcium-dependent domain containing 3	---	---	---
235324_at	-2.923	---	Transcribed locus	---	---	---
225747_at	-2.914	COQ10A	coenzyme Q10 homolog A (S. cerevisiae)	---	---	---
221039_s_at	-2.88	DDEF1	development and differentiation enhancing factor 1	---	regulation of ARF GTPase activity	GTPase activator activity /// protein binding /// protein binding /// ARF GTPase activator activity /// zinc ion binding /// metal ion binding
215554_at	-2.857	GPLD1	glycosylphosphatidylinositol specific phospholipase D1	---	---	glycosylphosphatidylinositol phospholipase D activity /// phospholipase D activity /// hydrolase activity
214828_s_at	-2.854	CTA-126B4.3 /// dJ222E13.2	OGI-96 protein /// similar to OGI-96	---	---	nucleic acid binding /// RNA binding
1561759_at	-2.847	LOC645513	Hypothetical LOC645513	---	cell cycle	GTP binding
1555763_x_at	-2.845	MKL1	Megakaryoblastic leukemia (translocation) 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	nucleotide binding /// nucleic acid binding /// RNA binding /// binding /// protein binding
238012_at	-2.843	DPP7	Dipeptidyl-peptidase 7	---	proteolysis	aminopeptidase activity /// serine-type endopeptidase activity /// protein binding /// peptidase activity /// serine-type peptidase activity /// serine-type peptidase activity /// hydrolase activity
235505_s_at	-2.841	---	MRNA full length insert cDNA clone EUROIMAGE 2362292	---	---	---
217967_s_at	-2.836	FAM129A	family with sequence similarity 129, member A	---	---	---
229513_at	-2.835	STRBP	Chromosome 9 open reading frame 45	---	multicellular organismal development /// spermatogenesis /// cell differentiation	DNA binding /// RNA binding /// double-stranded RNA binding
230454_at	-2.83	ICA1L	islet cell autoantigen 1, 69kDa-like	---	---	---
236512_at	-2.83	---	Transcribed locus	---	---	---
231283_at	-2.805	MGAT4A	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A	---	carbohydrate metabolic process /// N-glycan processing	alpha-1,3-mannosylglycoprotein 4-beta-N-acetylglucosaminyltransferase activity /// alpha-1,3-mannosylglycoprotein 4-beta-N-acetylglucosaminyltransferase activity /// transferase activity /// transferase activity, transferring glycosyl groups /// transferase activity, transferring hexosyl groups /// metal ion binding
1558301_a_at	-2.78	EFCAB5	EF-hand calcium binding domain 5	---	---	calcium ion binding
235121_at	-2.777	ZNF542	zinc finger protein 542	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
238089_at	-2.769	MAN2C1	mannosidase, alpha, class 2C, member 1	---	carbohydrate metabolic process /// mannose metabolic process /// metabolic process	alpha-mannosidase activity /// protein binding /// zinc ion binding /// hydrolase activity, acting on glycosyl bonds /// metal ion binding
235422_at	-2.756	---	Transcribed locus, strongly similar to XP_001125843.1 PREDICTED: hypothetical protein [Homo sapiens]	---	---	---
241721_at	-2.754	---	CDNA FLJ37844 fis, clone BRSSN2012622	---	---	---
226463_at	-2.728	ATP6V1C1	ATPase, H transporting, lysosomal 42kDa, V1 subunit C1	---	transport /// ion transport /// proton transport /// proton transport	transporter activity /// protein binding /// ATP binding /// hydrogen ion transmembrane transporter activity /// hydrolase activity /// hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances /// hydrogen ion transporting ATPase activity, rotational mechanism
243384_at	-2.707	---	Transcribed locus	---	---	---
232048_at	-2.702	FAM76B	family with sequence similarity 76, member B	---	---	---
229968_at	-2.696	---	Transcribed locus	---	---	---
229138_at	-2.688	PARP11	poly (ADP-ribose) polymerase family, member 11	---	---	NAD ADP-ribosyltransferase activity /// transferase activity /// transferase activity, transferring glycosyl groups
1558755_x_at	-2.685	ZNF763	zinc finger protein 763	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
214743_at	-2.671	CUX1	cut-like homeobox 1	---	negative regulation of transcription from RNA polymerase II promoter /// negative regulation of transcription from RNA polymerase II promoter /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// transport /// intra-Golgi vesicle-mediated transport /// multicellular organismal development /// multicellular organismal development /// lung development /// auditory receptor cell differentiation /// regulation of transcription	DNA binding /// chromatin binding /// transcription factor activity /// RNA polymerase II transcription factor activity /// transcription repressor activity /// sequence-specific DNA binding
1553612_at	-2.668	ZNF354B	zinc finger protein 354B	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
1559835_at	-2.667	---	Homo sapiens, clone IMAGE:5213378, mRNA	---	---	---
236056_s_at	-2.661	MARCH8	membrane-associated ring finger (C3HC4) 8	---	ubiquitin cycle /// immune response	protein binding /// zinc ion binding /// ligase activity /// metal ion binding
204075_s_at	-2.659	KIAA0562	KIAA0562	---	---	binding
223522_at	-2.655	C9orf45	chromosome 9 open reading frame 45	---	---	---
1558407_at	-2.637	PLEKHG2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2	---	regulation of Rho protein signal transduction	guanyl-nucleotide exchange factor activity /// Rho guanyl-nucleotide exchange factor activity
241818_at	-2.632	---	Transcribed locus	---	---	---
243848_at	-2.627	---	Transcribed locus	---	---	---
1553099_at	-2.607	TIGD1	tigger transposable element derived 1	---	regulation of transcription	nucleic acid binding /// DNA binding
226946_at	-2.605	C5orf33	chromosome 5 open reading frame 33	---	metabolic process	NAD kinase activity
232709_at	-2.603	---	CDNA FLJ13427 fis, clone PLACE1002477	---	---	---
234725_s_at	-2.594	SEMA4B	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4B	---	multicellular organismal development /// nervous system development /// cell differentiation	receptor activity
217911_s_at	-2.584	BAG3	BCL2-associated athanogene 3	Glycolysis_and_Gluconeogenesis	carbohydrate metabolic process /// glycolysis /// protein folding /// apoptosis /// anti-apoptosis /// metabolic process /// anaerobic glycolysis /// cellular carbohydrate metabolic process	catalytic activity /// L-lactate dehydrogenase activity /// L-lactate dehydrogenase activity /// binding /// protein binding /// oxidoreductase activity /// oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor /// Hsp70/Hsc70 protein regulator activity
219190_s_at	-2.58	EIF2C3 /// EIF2C4	eukaryotic translation initiation factor 2C, 3 /// eukaryotic translation initiation factor 2C, 4	---	translation /// gene silencing by RNA	nucleic acid binding /// translation initiation factor activity
203516_at	-2.578	SNTA1	syntrophin, alpha 1 (dystrophin-associated protein A1, 59kDa, acidic component)	---	muscle contraction	actin binding /// calcium ion binding /// protein binding /// protein binding /// calmodulin binding /// PDZ domain binding
243911_at	-2.57	---	Transcribed locus	---	---	---
209567_at	-2.561	RRS1	RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)	---	ribosome biogenesis and assembly	---
219810_at	-2.56	VCPIP1	valosin containing protein (p97)/p47 complex interacting protein 1	---	ubiquitin cycle /// ubiquitin cycle	ubiquitin-specific protease activity /// ubiquitin-specific protease activity /// peptidase activity /// cysteine-type peptidase activity /// hydrolase activity

201829_at	-2.552	NET1	neuroepithelial cell transforming gene 1	---	regulation of cell growth /// signal transduction /// intracellular signaling cascade /// regulation of Rho protein signal transduction	guanyl-nucleotide exchange factor activity /// guanyl-nucleotide exchange factor activity /// Rho guanyl-nucleotide exchange factor activity
228604_at	-2.529	---	CDNA FLJ41946 fis, clone PLACE6019701	---	---	---
231150_at	-2.525	---	Transcribed locus	---	---	---
241379_at	-2.523	C2orf13	chromosome 2 open reading frame 13	---	DNA repair /// response to DNA damage stimulus	protein binding /// zinc ion binding /// metal ion binding
226898_s_at	-2.52	SFPQ	Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)	---	DNA repair /// DNA recombination /// transcription /// regulation of transcription, DNA-dependent /// mRNA processing /// mRNA processing /// response to DNA damage stimulus /// RNA splicing /// RNA splicing	nucleotide binding /// nucleic acid binding /// DNA binding /// RNA binding /// protein binding
206663_at	-2.518	SP4	Sp4 transcription factor	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter	nucleic acid binding /// DNA binding /// RNA polymerase II transcription factor activity /// transcription coactivator activity /// zinc ion binding /// metal ion binding
1570505_at	-2.517	ABCB4	ATP-binding cassette, sub-family B (MDR/TAP), member 4	---	lipid metabolic process /// transport /// transport /// response to xenobiotic stimulus /// response to drug	nucleotide binding /// ATP binding /// xenobiotic-transporting ATPase activity /// hydrolase activity /// ATPase activity /// nucleoside-triphosphatase activity /// ATPase activity, coupled to transmembrane movement of substances /// ATPase activity, coupled to transmembrane movement of substances
218421_at	-2.512	CERK	ceramide kinase	---	ceramide metabolic process /// activation of protein kinase C activity	magnesium ion binding /// magnesium ion binding /// ceramide kinase activity /// ceramide kinase activity /// diacylglycerol kinase activity /// calcium ion binding /// kinase activity /// transferase activity
238658_at	-2.506	---	Transcribed locus	---	---	---
220141_at	-2.503	C11orf63	chromosome 11 open reading frame 63	---	---	---
239422_at	-2.503	GPC2	glypican 2	---	sulfur metabolic process /// cell-cell signaling /// biosynthetic process /// glycoprotein metabolic process /// oligosaccharide metabolic process /// proteoglycan biosynthetic process	galactosylceramide sulfotransferase activity /// transferase activity /// 3'-phosphoadenosine 5'-phosphosulfate binding /// galactose 3-O-sulfotransferase activity /// proteoglycan sulfotransferase activity
226486_at	-2.49	MTERFD2	MTERF domain containing 2	---	---	---
227637_at	-2.481	TFCP2	Transcription factor CP2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter	DNA binding /// DNA binding /// transcription factor activity
215547_at	-2.479	TSC22D2	TSC22 domain family, member 2	---	regulation of transcription, DNA-dependent	transcription factor activity
1565650_at	-2.468	---	---	---	---	---
1552634_a_at	-2.466	ZNF101	zinc finger protein 101	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
206199_at	-2.462	CEACAM7	carcinoembryonic antigen-related cell adhesion molecule 7	---	---	---
220338_at	-2.445	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	---	small GTPase mediated signal transduction	guanyl-nucleotide exchange factor activity
227335_at	-2.443	DIDO1	death inducer-obliterator 1	---	transcription /// apoptosis	protein binding /// zinc ion binding /// metal ion binding
1560094_at	-2.438	---	CDNA FLJ34740 fis, clone MESAN2008729	---	---	---
236282_at	-2.437	---	CDNA clone IMAGE:4826240	---	---	---
221908_at	-2.432	RNF12	ring finger protein, transmembrane 2	---	---	protein binding /// zinc ion binding /// metal ion binding
237138_at	-2.432	---	Transcribed locus	---	---	---
205316_at	-2.393	---	CDNA FLJ33407 fis, clone BRACE2010535	---	---	---
226806_s_at	-2.389	---	Transcribed locus	---	---	---
211967_at	-2.365	TMEM123	transmembrane protein 123	---	---	receptor activity
1553993_s_at	-2.353	MED25	mediator complex subunit 25	---	transcription /// regulation of transcription, DNA-dependent	---
239943_x_at	-2.337	---	MRNA; cDNA DKFZp667D1513 (from clone DKFZp667D1513)	---	---	---
214084_x_at	-2.326	LOC648998	similar to Neutrophil cytosol factor 1 (NCF-1) (Neutrophil NADPH oxidase factor 1) (47 kDa neutrophil oxidase factor) (p47-phox) (NCF-47K) (47 kDa autosomal chronic granulomatous disease protein) (NOXO2)	---	---	---
209907_s_at	-2.324	ITSN2	intersectin 2	---	endocytosis /// regulation of Rho protein signal transduction	SH3/SH2 adaptor activity /// Rho guanyl-nucleotide exchange factor activity /// calcium ion binding /// protein binding
216146_at	-2.311	---	Transcribed locus	---	---	---
243500_at	-2.297	CASD1	CAS1 domain containing 1	---	---	transferase activity
213827_at	-2.295	SNX26	sorting nexin 26	---	transport /// cell communication /// signal transduction /// protein transport	GTPase activator activity /// protein binding /// protein binding /// phosphoinositide binding
229575_at	-2.287	---	Transcribed locus	---	---	---
227131_at	-2.275	MAP3K3	mitogen-activated protein kinase kinase kinase 3	MAPK_Cascade	MAPKKK cascade /// protein amino acid phosphorylation /// protein kinase cascade /// positive regulation of I-kappaB kinase/NF-kappaB cascade /// protein amino acid autophosphorylation	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// MAP kinase kinase kinase activity /// MAP kinase kinase kinase activity /// signal transducer activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
222537_s_at	-2.272	CDC42SE1	CDC42 small effector 1	---	signal transduction	GTPase inhibitor activity /// protein binding
213054_at	-2.266	KIAA0841	KIAA0841	---	---	---
1553280_at	-2.243	PUS10	pseudouridylate synthase 10	---	tRNA processing	isomerase activity
204549_at	-2.243	IKBKE	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	Apoptosis_KEGG	protein amino acid phosphorylation /// protein amino acid phosphorylation /// immune response /// positive regulation of I-kappaB kinase/NF-kappaB cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// NF-kappaB-inducing kinase activity /// signal transducer activity /// protein binding /// ATP binding /// ATP binding /// IkappaB kinase activity /// kinase activity /// transferase activity
209894_at	-2.243	HLA-DRA	major histocompatibility complex, class II, DR alpha	---	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II /// immune response /// immune response /// antigen processing and presentation	MHC class II receptor activity
228754_at	-2.239	SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	---	amino acid metabolic process /// transport /// transport /// neurotransmitter transport /// taurine transport	neurotransmitter:sodium symporter activity /// taurine:sodium symporter activity /// taurine:sodium symporter activity /// symporter activity
218176_at	-2.23	MAGEF1	melanoma antigen family F, 1	---	---	---
209959_at	-2.212	NR4A3	nuclear receptor subfamily 4, group A, member 3	Hypertrophy_model	transcription /// regulation of transcription, DNA-dependent	DNA binding /// DNA binding /// transcription factor activity /// steroid hormone receptor activity /// steroid hormone receptor activity /// receptor activity /// ligand-dependent nuclear receptor activity /// thyroid hormone receptor activity /// binding /// zinc ion binding /// sequence-specific DNA binding /// metal ion binding
220202_s_at	-2.198	RC3H2	ring finger and CCCH-type zinc finger domains 2	---	---	nucleic acid binding /// DNA binding /// DNA binding /// protein binding /// zinc ion binding /// metal ion binding
240622_at	-2.196	---	---	---	---	---
226373_at	-2.194	SFXN5	sideroflexin 5	---	transport /// ion transport /// cation transport /// iron ion transport	iron ion binding /// cation transmembrane transporter activity
228433_at	-2.178	FLJ11236	hypothetical protein FLJ11236	---	---	---
212234_at	-2.158	ASXL1	additional sex combs like 1 (Drosophila)	---	transcription /// regulation of transcription, DNA-dependent	zinc ion binding /// metal ion binding
223903_at	-2.154	TLR9 /// TWF2	twinfilin, actin-binding protein, homolog 2 (Drosophila) /// toll-like receptor 9	---	inflammatory response /// immune response /// signal transduction /// response to virus /// defense response to bacterium /// positive regulation of interferon-gamma biosynthetic process /// innate immune response /// positive regulation of interferon-alpha biosynthetic process /// positive regulation of interferon-beta biosynthetic process	actin binding /// actin binding /// receptor activity /// transmembrane receptor activity /// protein binding /// protein binding /// ATP binding /// siRNA binding
240126_x_at	-2.144	---	Transcribed locus, strongly similar to XP_001125843.1 PREDICTED: hypothetical protein [Homo sapiens]	---	---	---
223797_at	-2.132	PRO2852	hypothetical protein PRO2852	---	---	---

205066_s_at	-2.127	ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1	---	ossification /// generation of precursor metabolites and energy /// phosphate metabolic process /// metabolic process /// nucleoside triphosphate catabolic process /// negative regulation of cell growth /// regulation of bone mineralization /// inorganic diphosphate transport /// cellular phosphate ion homeostasis /// sequestering of triacylglycerol /// negative regulation of protein amino acid autophosphorylation /// cellular response to insulin stimulus /// negative regulation of fat cell differentiation /// negative regulation of glycogen biosynthetic process /// negative regulation of glucose import /// negative regulation of insulin receptor signaling pathway /// 3'-phosphoadenosine 5'-phosphosulfate metabolic process	nucleic acid binding /// catalytic activity /// endonuclease activity /// phosphodiesterase I activity /// nucleotide diphosphatase activity /// nucleotide diphosphatase activity /// protein binding /// ATP binding /// hydrolase activity /// protein complex binding /// protein homodimerization activity /// metal ion binding /// nucleoside-triphosphate diphosphatase activity /// 3'-phosphoadenosine 5'-phosphosulfate binding
204542_at	-2.126	ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	---	protein amino acid glycosylation	sialyltransferase activity /// transferase activity /// transferase activity, transferring glycosyl groups
232866_at	-2.12	ZSCAN18	zinc finger and SCAN domain containing 18	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// zinc ion binding /// metal ion binding
1558306_at	-2.118	THADA	thyroid adenoma associated	---	transport	receptor activity /// transporter activity /// binding
233946_at	-2.109	SMU1	smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans)	---	---	---
218639_s_at	-2.105	ZXDC	ZXD family zinc finger C	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// protein binding /// zinc ion binding /// identical protein binding /// metal ion binding
238139_at	-2.102	---	Transcribed locus	---	---	---
215358_x_at	-2.1	LOC100129482 /// ZNF37B	zinc finger protein 37B /// similar to zinc finger protein 37a (KOX 21)	---	regulation of transcription, DNA-dependent	nucleic acid binding
229112_at	-2.092	LOC285813	hypothetical protein LOC285813	---	---	---
232441_at	-2.086	KRR1	KRR1, small subunit (SSU) processome component, homolog (yeast)	---	---	RNA binding /// protein binding
233855_at	-2.07	LOC284017	hypothetical protein LOC284017	---	---	---
200995_at	-2.068	IPO7	Importin 7	---	protein import into nucleus, docking /// transport /// intracellular protein transport /// signal transduction /// protein transport	small GTPase regulator activity /// transporter activity /// binding /// protein binding /// protein binding /// Ran GTPase binding /// protein transporter activity /// histone binding
1556006_s_at	-2.065	CSNK1A1	Casein kinase 1, alpha 1	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// Wnt receptor signaling pathway	nucleotide binding /// protein serine/threonine kinase activity /// casein kinase I activity /// ATP binding /// transferase activity
236202_at	-2.061	---	Transcribed locus	---	---	---
241190_at	-2.061	---	Transcribed locus	---	---	---
208615_s_at	-2.055	PTP4A2	protein tyrosine phosphatase type IVA, member 2	---	protein amino acid dephosphorylation /// dephosphorylation	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// prenylated protein tyrosine phosphatase activity /// protein binding /// hydrolase activity /// phosphoric monoester hydrolase activity
1563456_at	-2.046	LOC285026	hypothetical protein LOC285026	---	---	---
213899_at	-2.03	METAP2	methionyl aminopeptidase 2	---	proteolysis /// protein processing /// peptidyl-methionine modification /// N-terminal protein amino acid modification	aminopeptidase activity /// methionyl aminopeptidase activity /// methionyl aminopeptidase activity /// peptidase activity /// metalloexopeptidase activity /// hydrolase activity /// metal ion binding /// cobalt ion binding
32042_at	-2.024	ENOX2	ecto-NOX disulfide-thiol exchanger 2	---	regulation of cell growth /// transport /// ultradian rhythm /// cell growth /// rhythmic process /// oxidation reduction /// oxidation reduction	nucleotide binding /// nucleic acid binding /// copper ion binding /// protein disulfide oxidoreductase activity /// oxidoreductase activity
235580_at	-2.018	ZNF141	zinc finger protein 141	---	transcription /// regulation of transcription, DNA-dependent /// anatomical structure morphogenesis	nucleic acid binding /// DNA binding /// specific RNA polymerase II transcription factor activity /// zinc ion binding /// metal ion binding
1556498_at	-2.013	RPL5	ribosomal protein L5	---	translation /// translation	structural constituent of ribosome /// structural constituent of ribosome /// protein binding /// 5S rRNA binding /// rRNA binding
202124_s_at	-2.003	TRAK2	trafficking protein, kinesin binding 2	---	---	receptor binding /// protein binding /// GABA receptor binding
233852_at	-2.003	POLH	polymerase (DNA directed), eta	---	DNA replication /// DNA repair /// DNA repair /// regulation of DNA repair /// response to DNA damage stimulus	magnesium ion binding /// DNA binding /// damaged DNA binding /// DNA-directed DNA polymerase activity /// DNA-directed DNA polymerase activity /// transferase activity /// nucleotidyltransferase activity /// metal ion binding
240405_at	-1.999	---	Transcribed locus	---	---	---
237104_at	-1.998	---	Transcribed locus	---	---	---
220168_at	-1.994	CASC1	cancer susceptibility candidate 1	---	---	---
223930_at	-1.99	TOR1AIP1	torsin A interacting protein 1	---	---	---
241340_at	-1.99	---	Transcribed locus	---	---	---
223400_s_at	-1.97	PBRM1	polybromo 1	---	chromatin remodeling /// transcription /// regulation of transcription, DNA-dependent /// mitosis /// chromatin modification	DNA binding /// chromatin binding /// protein binding /// oxidoreductase activity
232778_at	-1.97	---	CDNA: FLJ22383 fis, clone HRC07564	---	---	---
209341_s_at	-1.969	IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	Apoptosis	protein modification process /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// positive regulation of NF-kappaB transcription factor activity	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein tyrosine kinase activity /// protein binding /// protein binding /// ATP binding /// ATP binding /// IkappaB kinase activity /// kinase activity /// transcription activator activity /// transferase activity /// identical protein binding
1564165_at	-1.967	PRKRIP1	PRKR interacting protein 1 (IL11 inducible)	---	---	---
204180_s_at	-1.957	ZBTB43	zinc finger and BTB domain containing 43	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// protein binding /// zinc ion binding /// metal ion binding
222482_at	-1.95	LOC100131851 /// LOC100134497 /// LOC401002 /// LOC646674 /// SSBP3	single stranded DNA binding protein 3 /// similar to single stranded DNA binding protein 3 /// hypothetical LOC646674 /// hypothetical protein LOC100131851 /// hypothetical protein LOC100134497	---	transcription /// regulation of transcription, DNA-dependent	DNA binding /// single-stranded DNA binding /// transcription regulator activity
200072_s_at	-1.949	HNRNPM	heterogeneous nuclear ribonucleoprotein M	---	nuclear mRNA splicing, via spliceosome /// mRNA processing /// RNA splicing	nucleotide binding /// nucleic acid binding /// RNA binding /// RNA binding /// receptor activity /// protein binding
1561871_at	-1.947	---	Homo sapiens, clone IMAGE:4300626, mRNA	---	---	---
238731_at	-1.947	SETDB2	SET domain, bifurcated 2	---	chromatin modification	DNA binding /// methyltransferase activity /// zinc ion binding /// transferase activity /// histone-lysine N-methyltransferase activity
222062_at	-1.944	IL27RA	interleukin 27 receptor, alpha	---	positive regulation of T-helper 1 type immune response /// negative regulation of T-helper 2 type immune response /// immune response /// cell surface receptor linked signal transduction /// positive regulation of interferon-gamma production /// regulation of isotype switching to IgG isotypes /// defense response to Gram-positive bacterium	receptor activity /// transmembrane receptor activity /// protein binding /// interleukin-27 receptor activity
206053_at	-1.94	ZNF510	zinc finger protein 510	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
223890_at	-1.936	LOC100132409	similar to PRO1082	---	---	---
218362_at	-1.926	RCBTB1	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1	---	transcription /// regulation of transcription, DNA-dependent /// cell cycle /// chromatin modification	protein binding
224051_at	-1.926	---	---	---	ubiquitin cycle /// amino acid and derivative metabolic process	nucleic acid binding /// peptidase activity /// cysteine-type peptidase activity /// zinc ion binding /// CoA hydrolase activity /// hydrolase activity /// metal ion binding
213339_at	-1.924	KIAA0495	KIAA0495	---	---	---
220767_at	-1.924	---	---	---	---	---
48808_at	-1.924	DHFR	dihydrofolate reductase	Nucleotide_Metabolism	glycine biosynthetic process /// glycine biosynthetic process /// one-carbon compound metabolic process /// nucleotide biosynthetic process /// nucleotide biosynthetic process	dihydrofolate reductase activity /// dihydrofolate reductase activity /// oxidoreductase activity
244741_s_at	-1.919	MGC9913	hypothetical protein MGC9913	---	---	---
201585_s_at	-1.902	SFPQ	splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)	---	DNA repair /// DNA recombination /// transcription /// regulation of transcription, DNA-dependent /// mRNA processing /// mRNA processing /// response to DNA damage stimulus /// RNA splicing /// RNA splicing	nucleotide binding /// nucleic acid binding /// DNA binding /// RNA binding /// protein binding
228996_at	-1.889	RC3H1	ring finger and CCCH-type zinc finger domains 1	---	---	nucleic acid binding /// RNA binding /// protein binding /// zinc ion binding /// metal ion binding

209710_at	-1.888	GATA2	GATA binding protein 2	---	cell fate determination /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// phagocytosis /// positive regulation of specific transcription from RNA polymerase II promoter /// pituitary gland development /// neuron differentiation /// cell maturation /// positive regulation of phagocytosis /// positive regulation of phagocytosis	DNA binding /// transcription factor activity /// transcription factor activity /// transcription factor activity /// protein binding /// protein binding /// transcription factor binding /// zinc ion binding /// sequence-specific DNA binding /// metal ion binding
236786_at	-1.888	---	Transcribed locus	---	---	---
215693_x_at	-1.886	DDX27	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	---	---	nucleotide binding /// nucleic acid binding /// helicase activity /// protein binding /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity
236326_at	-1.879	HDAC7	histone deacetylase 7	Cell_cycle_KEGG	transcription /// regulation of transcription, DNA-dependent /// inflammatory response /// nervous system development /// chromatin modification /// chromatin modification /// B cell differentiation /// B cell activation /// negative regulation of striated muscle development	histone deacetylase activity /// protein binding /// transcription factor binding /// specific transcriptional repressor activity /// hydrolase activity
242487_at	-1.875	CC2D1B	coiled-coil and C2 domain containing 1B	---	---	---
1568449_at	-1.854	---	MRNA activated in tumor suppression, clone TSAP18	---	---	---
209342_s_at	-1.852	IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	Apoptosis	protein modification process /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// positive regulation of NF-kappaB transcription factor activity	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein tyrosine kinase activity /// protein binding /// protein binding /// ATP binding /// ATP binding /// IkappaB kinase activity /// kinase activity /// transcription activator activity /// transferase activity /// identical protein binding
231022_at	-1.847	---	Transcribed locus	---	---	---
226660_at	-1.841	RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	G13_Signaling_Pathway /// Ribosomal_Proteins	protein amino acid phosphorylation /// signal transduction /// signal transduction	nucleotide binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// ATP binding /// kinase activity /// transferase activity
233200_at	-1.84	---	---	---	---	---
212479_s_at	-1.833	RMND5A	required for meiotic nuclear division 5 homolog A (S. cerevisiae)	---	---	---
232229_at	-1.829	SETX	senataxin	---	DNA repair /// double-strand break repair /// RNA processing /// response to DNA damage stimulus /// cell death	nucleotide binding /// DNA binding /// DNA helicase activity /// helicase activity /// ATP binding /// hydrolase activity
214902_x_at	-1.826	---	MRNA; cDNA DKFZp586A061 (from clone DKFZp586A061)	---	---	---
236286_at	-1.822	---	Transcribed locus	---	---	---
201447_at	-1.821	TIA1	TIA1 cytotoxic granule-associated RNA binding protein	---	apoptosis /// induction of apoptosis	nucleotide binding /// nucleic acid binding /// RNA binding /// poly(A) binding
210112_at	1.816	HPS1	Hermansky Pudlak syndrome 1		lysosome organization and biogenesis /// visual perception /// response to stimulus	protein binding /// protein dimerization activity
219974_x_at	-1.814	ECHDC1	enoyl Coenzyme A hydratase domain containing 1	---	metabolic process	catalytic activity /// isomerase activity
218190_s_at	-1.798	UCRC	ubiquinol-cytochrome c reductase complex (7.2 kD)	Electron_Transport_Chain	mitochondrial electron transport, ubiquinol to cytochrome c /// mitochondrial electron transport, ubiquinol to cytochrome c /// transport /// oxidation reduction	ubiquinol-cytochrome-c reductase activity /// ubiquinol-cytochrome-c reductase activity
213705_at	-1.795	---	CDNA FLJ30007 fis, clone 3NB692000012	---	---	---
221787_at	-1.791	C6orf120	chromosome 6 open reading frame 120	---	---	---
222046_at	-1.789	ARS2	arsenate resistance protein 2	---	response to arsenic	protein binding
213821_s_at	-1.784	---	Clone 488469 iduronate-2-sulfatase (IDS2) pseudogene, mRNA sequence	---	---	---
1569112_at	-1.778	SLC44A5	solute carrier family 44, member 5	---	---	---
1560724_at	-1.777	---	CDNA FLJ33564 fis, clone BRAMY2010135	---	---	---
1563128_at	-1.766	---	Homo sapiens, clone IMAGE:4272979	---	---	---
205030_at	-1.765	FABP7	fatty acid binding protein 7, brain	---	fatty acid metabolic process /// transport /// nervous system development /// negative regulation of cell proliferation	transporter activity /// binding /// lipid binding /// lipid binding
231714_s_at	-1.757	AP4B1	adaptor-related protein complex 4, beta 1 subunit	---	protein complex assembly /// transport /// intracellular protein transport /// protein transport /// vesicle-mediated transport	transporter activity /// binding /// protein binding /// protein binding
204807_at	-1.756	TMEM5	transmembrane protein 5	---	---	---
201778_s_at	-1.754	KIAA0494	KIAA0494	---	---	calcium ion binding
236301_at	-1.745	---	Full length insert cDNA clone YY82H04	---	---	---
237374_at	-1.74	---	Transcribed locus, strongly similar to XP_857309.1 PREDICTED: similar to poly(rC) binding protein 2 isoform 3 [Canis familiaris]	---	---	---
207336_at	-1.723	SOX5	SRY (sex determining region Y)-box 5	---	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter	DNA binding /// transcription factor activity
220939_s_at	-1.723	DPP8	dipeptidyl-peptidase 8	---	proteolysis /// proteolysis /// immune response	aminopeptidase activity /// serine-type endopeptidase activity /// dipeptidyl-peptidase IV activity /// peptidase activity /// serine-type peptidase activity /// dipeptidyl-peptidase activity /// hydrolase activity
201939_at	-1.711	PLK2	polo-like kinase 2 (Drosophila)	---	mitotic cell cycle /// protein amino acid phosphorylation /// positive regulation of I-kappaB kinase/NF-kappaB cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// signal transducer activity /// protein binding /// ATP binding /// kinase activity /// transferase activity
1555892_s_at	-1.71	LOC253039	hypothetical LOC253039	---	---	---
236665_at	-1.705	CCDC18	coiled-coil domain containing 18	---	---	---
203273_s_at	-1.692	TUSC2	tumor suppressor candidate 2	---	cell cycle /// cell-cell signaling /// cell proliferation /// negative regulation of cell cycle	protein binding
222618_at	-1.671	SMU1	smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans)	---	---	---
207535_s_at	-1.665	NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	---	follicular dendritic cell differentiation /// germinal center formation /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// signal transduction /// extracellular matrix organization and biogenesis /// regulation of transcription /// spleen development	DNA binding /// transcription factor activity /// transcription factor activity /// transcription coactivator activity /// protein binding /// protein binding
224542_s_at	-1.663	NFATC2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// regulation of transcription /// positive regulation of transcription	DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// protein binding /// transcription activator activity
214232_at	-1.647	---	---	---	---	---
223296_at	-1.642	SLC25A33	solute carrier family 25, member 33	---	transport	binding
213195_at	-1.638	LOC201229	hypothetical protein LOC201229	---	---	---
208196_x_at	-1.635	NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// intracellular signaling cascade /// regulation of transcription	DNA binding /// transcription factor activity /// transcription factor activity /// FK506 binding /// transcription activator activity
239333_x_at	-1.63	---	CDNA FLJ30541 fis, clone BRAWH2001355	---	---	---
201190_s_at	-1.627	PITPNA	phosphatidylinositol transfer protein, alpha	---	lipid metabolic process /// transport /// visual perception	protein binding /// phospholipid binding /// lipid binding /// phosphatidylcholine transmembrane transporter activity /// phosphatidylinositol transporter activity
226574_at	-1.626	PSPC1	paraspeckle component 1	---	transcription /// regulation of transcription, DNA-dependent	nucleotide binding /// nucleic acid binding /// RNA binding
221709_s_at	-1.623	C14orf131	chromosome 14 open reading frame 131	---	---	nucleic acid binding /// zinc ion binding /// metal ion binding
222070_at	-1.621	DND1	dead end homolog 1 (zebrafish)	---	multicellular organismal development	nucleotide binding /// nucleic acid binding /// RNA binding
225916_at	-1.615	ZNF131	zinc finger protein 131	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// protein binding /// zinc ion binding /// metal ion binding
202159_at	-1.614	FARSA	phenylalanyl-tRNA synthetase, alpha subunit	---	translation /// tRNA aminoacylation for protein translation /// phenylalanyl-tRNA aminoacylation	nucleotide binding /// aminoacyl-tRNA ligase activity /// phenylalanine-tRNA ligase activity /// phenylalanine-tRNA ligase activity /// ATP binding /// ligase activity
212323_s_at	-1.614	VPS13D	vacuolar protein sorting 13 homolog D (S. cerevisiae)	---	protein localization	---
231085_s_at	-1.611	---	---	---	---	---
1566798_at	-1.607	SLC35E1	solute carrier family 35, member E1	---	transport	---
1553318_at	-1.606	RIBC1	RIB43A domain with coiled-coils 1	---	---	---

217761_at	-1.597	ADI1	acireductone dioxygenase 1	---	amino acid biosynthetic process /// methionine biosynthetic process /// methionine salvage	protein binding /// nickel ion binding /// oxidoreductase activity /// oxidoreductase activity /// oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen /// metal ion binding
208830_s_at	-1.594	SUPT6H	suppressor of Ty 6 homolog (S. cerevisiae)	---	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process /// chromatin remodeling /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter	transcription factor activity /// transcription elongation regulator activity /// RNA binding /// protein binding /// hydrolase activity, acting on ester bonds
217921_at	-1.592	MAN1A2	mannosidase, alpha, class 1A, member 2	---	N-glycan processing /// metabolic process	mannosyl-oligosaccharide 1,2-alpha-mannosidase activity /// mannosyl-oligosaccharide 1,2-alpha-mannosidase activity /// calcium ion binding /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds
226562_at	-1.591	ZSCAN29	zinc finger and SCAN domain containing 29	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// zinc ion binding /// metal ion binding
220961_s_at	-1.577	TBRG4	transforming growth factor beta regulator 4	---	G1 phase of mitotic cell cycle /// apoptosis /// cell cycle arrest /// positive regulation of cell proliferation	protein kinase activity /// protein binding /// ATP binding
202082_s_at	-1.576	SEC14L1	SEC14-like 1 (S. cerevisiae)	---	transport	transporter activity /// binding
217688_at	-1.569	CATSPER2 /// CATSPER2P1	cation channel, sperm associated 2 /// cation channel, sperm associated 2 pseudogene 1	---	transport /// ion transport /// calcium ion transport /// multicellular organismal development /// spermatogenesis /// cell differentiation	ion channel activity /// voltage-gated ion channel activity /// calcium channel activity /// calcium ion binding
1569105_at	-1.568	SETD5	SET domain containing 5	---	---	---
210232_at	-1.56	CDC42	cell division cycle 42 (GTP binding protein, 25kDa)	G13_Signaling_Pathway /// Integrin-mediated_cell_adhesion_KEGG	nuclear migration /// establishment and/or maintenance of cell polarity /// small GTPase mediated signal transduction /// actin cytoskeleton organization and biogenesis /// macrophage differentiation /// positive regulation of pseudopodium formation /// negative regulation of protein complex assembly /// positive regulation of phosphoinositide 3-kinase activity /// filopodium formation /// cell division	nucleotide binding /// GTPase activity /// protein binding /// GTP binding /// GTP-dependent protein binding
219131_at	-1.555	UBIAD1	UbiA prenyltransferase domain containing 1	---	---	prenyltransferase activity
229783_at	-1.552	LOC728163	hypothetical protein LOC728163	---	---	---
208151_x_at	-1.549	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	---	RNA processing	nucleotide binding /// nucleic acid binding /// RNA binding /// RNA binding /// RNA helicase activity /// helicase activity /// ATP binding /// ATP-dependent helicase activity /// RNA-dependent ATPase activity /// hydrolase activity
204004_at	-1.546	PAWR	PRKC, apoptosis, WT1, regulator	---	negative regulation of transcription from RNA polymerase II promoter /// transcription /// regulation of transcription, DNA-dependent /// apoptosis /// apoptosis /// negative regulation of cell proliferation /// positive regulation of apoptosis	transcription corepressor activity /// protein binding /// enzyme binding
204538_x_at	-1.533	NPIP	nuclear pore complex interacting protein	---	transport /// neuropeptide signaling pathway /// protein transport /// mRNA transport /// intracellular protein transport across a membrane	---
202569_s_at	-1.529	MARK3	MAP/microtubule affinity-regulating kinase 3	---	protein amino acid phosphorylation	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein binding /// ATP bindin /// kinase activity /// transferase activity
210882_s_at	-1.523	TRO	trophinin	---	cell adhesion /// homophilic cell adhesion /// embryo implantation	protein binding
216136_at	-1.515	---	Transcribed locus	---	---	---
204361_s_at	-1.511	SKAP2	src kinase associated phosphoprotein 2	---	protein complex assembly /// signal transduction /// negative regulation of cell proliferation /// B cell activation	SH3/SH2 adaptor activity
210092_at	-1.509	MAGOH	mago-nashi homolog, proliferation-associated (Drosophila)	---	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay /// mRNA processing /// transport /// RNA splicing /// mRNA transport	RNA binding /// protein binding
1554616_at	-1.468	SERPINB8	serpin peptidase inhibitor, clade B (ovalbumin), member 8	---	---	endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity /// protein binding
237019_at	-1.461	---	Transcribed locus	---	---	---
240768_x_at	-1.438	---	Transcribed locus	---	---	---
202050_s_at	-1.429	ZMYM4	zinc finger, MYM-type 4	---	multicellular organismal development	DNA binding /// zinc ion binding /// metal ion binding
210790_s_at	-1.411	SAR1A	SAR1 gene homolog A (S. cerevisiae)	---	transport /// intracellular protein transport /// ER to Golgi vesicle-mediated transport /// small GTPase mediated signal transduction /// protein transport /// vesicle-mediated transport	nucleotide binding /// GTPase activity /// GTP binding
220619_at	-1.407	CHD7	chromodomain helicase DNA binding protein 7	---	blood vessel development /// in utero embryonic development /// heart morphogenesis /// chromatin assembly or disassembly /// transcription /// regulation of transcription, DNA-dependent /// adult heart development /// sensory perception of sound /// locomotory behavior /// adult walking behavior /// blood circulation /// chromatin modification /// female genitalia development /// embryonic hindlimb morphogenesis /// positive regulation of multicellular organism growth /// inner ear morphogenesis /// camera-type eye development /// nose development /// palate development	nucleotide binding /// nucleic acid binding /// DNA binding /// chromatin binding /// helicase activity /// ATP binding /// hydrolase activity
1569192_at	-1.396	---	Transcribed locus	---	---	---
213482_at	-1.395	DOCK3	dedicator of cytokinesis 3	---	---	guanyl-nucleotide exchange factor activity /// protein binding /// GTP binding /// GTPase binding
1560659_at	-1.391	---	---	---	---	---
229272_at	-1.389	FNBP4	formin binding protein 4	---	cell adhesion /// G-protein coupled receptor protein signaling pathway	histamine receptor activity /// structural molecule activity /// protein binding
213998_s_at	-1.381	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	---	RNA processing	nucleotide binding /// nucleic acid binding /// RNA binding /// RNA binding /// RNA helicase activity /// helicase activity /// ATP binding /// ATP-dependent helicase activity /// RNA-dependent ATPase activity /// hydrolase activity
219199_at	-1.373	AFF4	AF4/FMR2 family, member 4	---	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter	transcription factor activity
214959_s_at	-1.368	API5	apoptosis inhibitor 5	---	apoptosis /// anti-apoptosis /// anti-apoptosis /// anti-apoptosis /// anti-apoptosis /// anti-apoptosis	binding /// fibroblast growth factor binding
1554793_at	-1.362	UBE3C	ubiquitin protein ligase E3C	---	protein polyubiquitination /// protein modification process /// ubiquitin cycle	ubiquitin-protein ligase activity /// ubiquitin-protein ligase activity /// protein binding /// ligase activity
236163_at	-1.345	MOBKL2A	MOB1, Mps One Binder kinase activator-like 2A (yeast)	---	---	protein binding /// zinc ion binding /// metal ion binding
214540_at	-1.322	HIST1H2BC /// HIST1H2BE /// HIST1H2BF /// HIST1H2BG /// HIST1H2BI /// HIST1H2BO	histone cluster 1, H2bg /// histone cluster 1, H2bf /// histone cluster 1, H2be /// histone cluster 1, H2bi /// histone cluster 1, H2bc /// histone cluster 1, H2bo	---	nucleosome assembly /// nucleosome assembly /// defense response to bacterium	DNA binding /// DNA binding /// protein binding
210932_s_at	-1.313	RNF6	ring finger protein (C3H2C3 type) 6	---	---	protein binding /// zinc ion binding /// zinc ion binding /// metal io binding
225780_at	-1.305	RSC1A1	regulatory solute carrier protein, family 1, member 1	---	transcription /// regulation of transcription, DNA-dependent /// dephosphorylation /// negative regulation of transport	protein tyrosine phosphatase activity /// ion channel inhibitor activity /// phosphoric monoester hydrolase activity
206192_at	-1.302	MAP3K14	mitogen-activated protein kinase kinase kinase 14	Apoptosis_GenMAPP	protein amino acid phosphorylation	nucleotide binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// MAP kinase kinase kinase activity /// protein binding /// ATP binding /// kinase activity /// transferase activity
214900_at	-1.3	ZKSCAN1	zinc finger with KRAB and SCAN domains 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// transcription factor activity /// zinc ion binding /// meta ion binding
233806_at	-1.28	---	CDNA FLJ10073 fis, clone HEMBA1001731	---	---	---
239198_at	-1.271	EZH1	enhancer of zeste homolog 1 (Drosophila)	---	transcription /// regulation of transcription, DNA-dependent /// anatomical structure morphogenesis	DNA binding /// chromatin binding /// protein binding
212486_s_at	-1.261	FYN	FYN oncogene related to SRC, FGR, YES	Integrin-mediated_cell_adhesion_KEGG	protein amino acid phosphorylation /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// calcium ion transport /// protein kinase cascade /// multicellular organismal development /// learning /// feeding behavior /// T cell receptor signaling pathway	nucleotide binding /// protein kinase activity /// protein tyrosine kinase activity /// protein tyrosine kinase activity /// non-membrane spanning protein tyrosine kinase activity /// non-membrane spanning protein tyrosine kinase activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity /// manganese ion binding /// identical protein binding /// metal ion binding

9.2. Genes exclusively regulated in memory B cells upon EBV transformation

236931_at	-1.437	---	---	---	---	---
219605_at	-1.433	ZNF3	zinc finger protein 3	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// multicellular organismal development /// cell differentiation /// leukocyte activation	nucleic acid binding /// DNA binding /// DNA binding /// transcription factor activity /// zinc ion binding /// zinc ion binding /// identical protein binding /// metal ion binding
224281_s_at	-1.426	NGRN	neugrin, neurite outgrowth associated	---	multicellular organismal development /// nervous system development /// cell differentiation /// neuron differentiation	---
211905_s_at	-1.423	ITGB4	integrin, beta 4	Integrin-mediated_cell_adhesion_KEGG	cell communication /// cell adhesion /// cell adhesion /// cell-matrix adhesion /// integrin-mediated signaling pathway /// multicellular organismal development	receptor activity /// binding /// protein binding /// protein binding
218548_x_at	-1.411	TEX264	testis expressed 264	---	---	---
225044_at	-1.398	NT5C3L	5'-nucleotidase, cytosolic III-like	---	nucleotide metabolic process	nucleotide binding /// magnesium ion binding /// 5'-nucleotidase activity /// transferase activity /// hydrolase activity /// metal ion binding
201545_s_at	-1.389	PABPN1	poly(A) binding protein, nuclear 1	mRNA_processing_Reactome	RNA processing /// mRNA processing /// muscle contraction	nucleotide binding /// nucleic acid binding /// RNA binding /// RNA binding /// protein binding
238433_at	-1.382	SNX5	sorting nexin 5	---	transport /// cell communication /// protein transport	protein binding /// phosphoinositide binding
236431_at	-1.377	SR140	U2-associated SR140 protein	---	RNA processing	nucleotide binding /// nucleic acid binding /// RNA binding
226075_at	-1.369	SPSB1	splA/ryanodine receptor domain and SOCS box containing 1	---	ubiquitin cycle /// intracellular signaling cascade	receptor activity
207065_at	-1.366	KRT75	keratin 75	---	---	structural molecule activity /// structural molecule activity
203580_s_at	-1.361	SLC7A6 /// TRPV6	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6 /// transient receptor potential cation channel, subfamily V, member 6	---	protein complex assembly /// amino acid metabolic process /// transport /// transport /// ion transport /// calcium ion transport /// calcium ion transport /// amino acid transport /// regulation of calcium ion-dependent exocytosis	DNA binding /// receptor activity /// ion channel activity /// calcium channel activity /// calcium channel activity /// calcium ion binding /// calmodulin binding /// calmodulin binding /// amino acid transmembrane transporter activity /// amino acid transmembrane transporter activity
227479_at	-1.361	KIAA1244	KIAA1244	---	regulation of ARF protein signal transduction	guanyl-nucleotide exchange factor activity /// ARF guanyl-nucleotide exchange factor activity
236686_at	-1.358	---	Transcribed locus	---	---	---
1561813_at	-1.354	---	MRNA full length insert cDNA clone EUROIMAGE 200999	---	---	---
215600_x_at	-1.348	FBXW12	F-box and WD repeat domain containing 12	---	ubiquitin cycle	---
214019_at	-1.346	---	---	G1_to_S_cell_cycle_Reactome /// Wnt_signaling	G1/S transition of mitotic cell cycle /// re-entry into mitotic cell cycle /// positive regulation of protein amino acid phosphorylation /// protein amino acid phosphorylation /// cell cycle /// response to iron ion /// unfolded protein response /// fat cell differentiation /// positive regulation of cyclin-dependent protein kinase activity /// cell division /// response to calcium ion	protein kinase activity /// protein binding /// protein binding /// cyclin-dependent protein kinase regulator activity /// protein kinase binding /// protein kinase binding
217735_s_at	-1.34	EIF2AK1	eukaryotic translation initiation factor 2-alpha kinase 1	Translation_Factors	translation /// regulation of translation /// protein amino acid phosphorylation /// response to stress /// negative regulation of cell proliferation /// response to external stimulus /// negative regulation of translation /// negative regulation of translational initiation by iron /// protein amino acid autophosphorylation /// protein amino acid autophosphorylation /// negative regulation of hemoglobin biosynthetic process /// negative regulation of hemoglobin biosynthetic process	nucleotide binding /// damaged DNA binding /// translation initiation factor activity /// protein kinase activity /// protein serine/threonine kinase activity /// eukaryotic translation initiation factor 2alpha kinase activity /// protein binding /// ATP binding /// kinase activity /// transferase activity /// heme binding /// heme binding /// protein homodimerization activity /// protein homodimerization activity
1560386_at	-1.329	---	Homo sapiens, clone IMAGE:5740472, mRNA	---	---	---
215310_at	-1.311	APC	adenomatous polyposis coli	Wnt_signaling	protein complex assembly /// response to DNA damage stimulus /// negative regulation of microtubule depolymerization /// cell cycle /// cell cycle arrest /// cell adhesion /// negative regulation of cell proliferation /// Wnt receptor signaling pathway /// negative regulation of cyclin-dependent protein kinase activity /// negative regulation of cell cycle /// regulation of attachment of spindle microtubules to kinetochore /// Wnt receptor signaling pathway through beta-catenin /// Wnt receptor signaling pathway through beta-catenin	binding /// protein binding /// protein binding /// beta-catenin binding /// beta-catenin binding /// microtubule binding /// microtubule binding /// protein kinase CK2 regulator activity /// protein kinase binding
214728_x_at	-1.276	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	---	negative regulation of transcription from RNA polymerase II promoter /// blastocyst growth /// blastocyst hatching /// methylation-dependent chromatin silencing /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// glial cell fate determination /// forebrain development /// hindbrain development	nucleotide binding /// nucleic acid binding /// DNA binding /// chromatin binding /// transcription factor activity /// transcription coactivator activity /// helicase activity /// helicase activity /// protein binding /// ATP binding /// transcription factor binding /// hydrolase activity /// identical protein binding /// protein N-terminus binding
218317_x_at	-1.273	GIYD1 /// GIYD2	GIY-YIG domain containing 2 /// GIY-YIG domain containing 1	---	DNA repair	nuclease activity
233282_at	-1.215	---	CDNA FLJ13333 fis, clone OVARC1001828	---	---	---
204027_s_at	-1.187	METTL1	methyltransferase like 1	---	tRNA modification /// tRNA processing	methyltransferase activity /// methyltransferase activity /// tRNA (guanine-N7-)-methyltransferase activity /// tRNA (guanine-N7-)-methyltransferase activity /// transferase activity
1561867_at	-1.15	---	Full length insert cDNA clone YR85B05	---	---	---
233532_x_at	-1.109	IFT52	intraflagellar transport 52 homolog (Chlamydomonas)	---	neural tube formation /// heart looping /// smoothened signaling pathway /// determination of left/right symmetry /// dorsal/ventral pattern formation /// embryonic digit morphogenesis /// positive regulation of proteolysis	---

GENES UPREGULATED EXCLUSIVELY IN MEMORY B CELLS UPON TRANSFORMATION BY EBV

Probe Set ID	Fold Change	Gene Symbol	Gene Title	Pathway	go biological process term	go molecular function term
201667_at	129.9	GJA1	gap junction protein, alpha 1, 43kDa	Calcium_regulation_in_cardiac_cells /// Smooth_muscle_contraction	in utero embryonic development /// neuron migration /// heart looping /// epithelial cell maturation /// transport /// apoptosis /// muscle contraction /// cell communication /// cell-cell signaling /// cell-cell signaling /// heart development /// adult heart development /// sensory perception of sound /// regulation of heart contraction /// negative regulation of cell proliferation /// response to pH /// vascular transport /// ATP transport /// gap junction assembly /// embryonic heart tube development /// positive regulation of I-kappaB kinase/NF-kappaB cascade /// skeletal muscle regeneration /// positive regulation of protein catabolic process /// positive regulation of striated muscle development /// blood vessel morphogenesis /// neurite morphogenesis /// protein oligomerization /// regulation of calcium ion transport	signal transducer activity /// gap junction channel activity /// protein binding /// protein binding /// ion transmembrane transporter activity /// SH3 domain binding /// PDZ domain binding
227140_at	109.6	---	CDNA FLJ11041 fis, clone PLACE1004405	---	---	---
226545_at	85.94	CD109	CD109 molecule	---	---	endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity /// protein binding /// wide-spectrum protease inhibitor activity
201012_at	83.01	ANXA1	annexin A1	Prostaglandin_synthesis_regulation	lipid metabolic process /// anti-apoptosis /// cell motility /// inflammatory response /// cell cycle /// signal transduction /// signal transduction /// cell surface receptor linked signal transduction /// peptide cross-linking /// keratinocyte differentiation /// regulation of cell proliferation /// arachidonic acid secretion	phospholipase inhibitor activity /// phospholipase inhibitor activity /// receptor binding /// structural molecule activity /// calcium ion binding /// calcium ion binding /// protein binding /// protein binding /// phospholipid binding /// calcium-dependent phospholipid binding /// phospholipase A2 inhibitor activity /// protein binding, bridging
202766_s_at	63.83	FBN1	fibrillin 1	---	skeletal development /// heart development /// blood coagulation	transmembrane receptor activity /// extracellular matrix structural constituent /// extracellular matrix structural constituent /// extracellular matrix structural constituent /// binding /// calcium ion binding /// calcium ion binding /// calcium ion binding /// protein binding
221127_s_at	61.73	RIG	regulated in glioma	---	---	---
214247_s_at	45.97	DKK3	dickkopf homolog 3 (Xenopus laevis)	---	multicellular organismal development /// anatomical structure morphogenesis /// Wnt receptor signaling pathway /// negative regulation of Wnt receptor signaling pathway	endopeptidase inhibitor activity
210987_x_at	42.17	TPM1	tropomyosin 1 (alpha)	Striated_muscle_contraction	cell motility /// regulation of muscle contraction /// regulation of heart contraction	actin binding /// structural constituent of cytoskeleton /// structural constituent of muscle
205669_at	35.49	LAMP3	lysosomal-associated membrane protein 3	---	cell proliferation	---
204627_s_at	34.42	ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	Integrin-mediated_cell_adhesion_KEGG	cell-substrate junction assembly /// cell adhesion /// cell adhesion /// cell-matrix adhesion /// integrin-mediated signaling pathway /// multicellular organismal development /// blood coagulation /// regulation of cell migration	receptor activity /// integrin binding /// binding /// protein binding /// protein binding /// identical protein binding
208636_at	31.37	ACTN1	actinin, alpha 1	---	regulation of apoptosis /// focal adhesion formation /// actin filament bundle formation /// negative regulation of cell motility	actin binding /// integrin binding /// calcium ion binding /// protein binding /// protein binding /// vinculin binding /// actin filament binding
201615_x_at	29.75	CALD1	caldesmon 1	---	cell motility /// muscle contraction	actin binding /// actin binding /// calmodulin binding /// calmodulin binding /// tropomyosin binding /// myosin binding
209191_at	27.79	TUBB6	tubulin, beta 6	---	microtubule-based process /// microtubule-based movement /// protein polymerization	nucleotide binding /// GTPase activity /// structural molecule activity /// GTP binding
202381_at	25.32	ADAM9	ADAM metallopeptidase domain 9 (meltrin gamma)	---	proteolysis /// protein kinase cascade	metalloendopeptidase activity /// integrin binding /// protein binding /// peptidase activity /// metallopeptidase activity /// metallopeptidase activity /// metallopeptidase activity /// zinc ion binding /// hydrolase activity /// SH3 domain binding /// protein kinase binding /// metal ion binding
200665_s_at	23.14	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	---	ossification /// transmembrane receptor protein tyrosine kinase signaling pathway	nucleotide binding /// motor activity /// actin binding /// actin binding /// copper ion binding /// calcium ion binding /// calcium ion binding /// calmodulin binding /// calmodulin binding /// collagen binding /// ATP binding /// ATP binding
202336_s_at	22.27	PAM	peptidylglycine alpha-amidating monooxygenase	---	peptide amidation /// protein modification process /// peptide metabolic process /// peptide metabolic process /// mitotic chromosome condensation /// cellular process	chromatin binding /// catalytic activity /// monooxygenase activity /// peptidylglycine monooxygenase activity /// peptidylglycine monooxygenase activity /// peptidylglycine monooxygenase activity /// peptidylamidoglycolate lyase activity /// copper ion binding /// protein binding /// zinc ion binding /// oxidoreductase activity /// lyase activity /// L-ascorbic acid binding /// metal ion binding
201631_s_at	21.79	IER3	immediate early response 3	---	apoptosis /// anti-apoptosis /// anti-apoptosis /// anatomical structure morphogenesis	protein binding
203695_s_at	20.85	DFNA5	deafness, autosomal dominant 5	---	sensory perception of sound /// sensory perception of sound /// inner ear receptor cell differentiation	---
202552_s_at	20.2	CRIM1	cysteine rich transmembrane BMP regulator 1 (chordin-like)	---	regulation of cell growth /// proteolysis /// nervous system development	cysteine-type endopeptidase activity /// enzyme inhibitor activity /// serine-type endopeptidase inhibitor activity /// insulin-like growth factor receptor activity /// insulin-like growth factor binding
212353_at	17.21	SULF1	sulfatase 1	---	apoptosis /// metabolic process /// heparan sulfate proteoglycan metabolic process	catalytic activity /// arylsulfatase activity /// calcium ion binding /// sulfuric ester hydrolase activity /// hydrolase activity /// metal ion binding
212977_at	14.44	CXCR7	chemokine (C-X-C motif) receptor 7	GPCRDB_Class_A_Rhodopsin-like /// Smooth_muscle_contraction	signal transduction /// G-protein coupled receptor protein signaling pathway	rhodopsin-like receptor activity /// signal transducer activity /// receptor activity /// G-protein coupled receptor activity
209921_at	13.46	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	---	protein complex assembly /// transport /// amino acid transport	amino acid transmembrane transporter activity /// cystine:glutamate antiporter activity
218864_at	12.9	DSE	dermatan sulfate epimerase	---	dermatan sulfate biosynthetic process	isomerase activity /// chondroitin-glucuronate 5-epimerase activity /// chondroitin-glucuronate 5-epimerase activity
204284_at	12.36	PPP1R3C	protein phosphatase 1, regulatory (inhibitor) subunit 3C	Circadian_Exercise	carbohydrate metabolic process /// glycogen metabolic process /// glycogen biosynthetic process	protein serine/threonine phosphatase activity
214720_x_at	12.32	Sep 10	Septin 10	---	cell cycle /// cell division	nucleotide binding /// protein binding /// GTP binding
208370_s_at	12.16	RCAN1	regulator of calcineurin 1	---	signal transduction /// central nervous system development /// blood circulation /// calcium-mediated signaling	DNA binding /// transcription factor activity /// protein binding
200771_at	12.04	LAMC1	laminin, gamma 1 (formerly LAMB2)	Inflammatory_Response_Pathway	protein complex assembly /// cell adhesion /// cell adhesion /// endoderm development /// cell migration /// extracellular matrix disassembly /// hemidesmosome assembly /// positive regulation of epithelial cell proliferation	extracellular matrix structural constituent /// protein binding
225018_at	11.98	SPIRE1	spire homolog 1 (Drosophila)	---	transport	actin binding /// zinc ion binding
212958_x_at	11.85	PAM	peptidylglycine alpha-amidating monooxygenase	---	peptide amidation /// protein modification process /// peptide metabolic process /// peptide metabolic process /// mitotic chromosome condensation /// cellular process	chromatin binding /// catalytic activity /// monooxygenase activity /// peptidylglycine monooxygenase activity /// peptidylglycine monooxygenase activity /// peptidylglycine monooxygenase activity /// peptidylamidoglycolate lyase activity /// copper ion binding /// protein binding /// zinc ion binding /// oxidoreductase activity /// lyase activity /// L-ascorbic acid binding /// metal ion binding
205767_at	11.61	EREG	epiregulin	---	angiogenesis /// ovarian cumulus expansion /// ovarian cumulus expansion /// oocyte maturation /// oocyte maturation /// positive regulation of cytokine production /// positive regulation of cytokine production /// female meiosis /// female meiosis /// epidermal growth factor receptor signaling pathway /// epidermal growth factor receptor signaling pathway /// cell-cell signaling /// multicellular organismal development /// cell proliferation /// positive regulation of cell proliferation /// positive regulation of cell proliferation /// negative regulation of cell proliferation /// negative regulation of cell proliferation /// mRNA transcription /// anatomical structure morphogenesis /// organ morphogenesis /// negative regulation of transcription /// cytokine and chemokine mediated signaling pathway /// cell differentiation /// keratinocyte differentiation /// ovulation /// ovulation /// wound healing /// positive regulation of cytokine biosynthetic process /// positive regulation of phosphorylation /// positive regulation of phosphorylation /// luteinizing hormone signaling pathway /// luteinizing hormone signaling pathway ///	epidermal growth factor receptor binding /// epidermal growth factor receptor binding /// epidermal growth factor receptor binding /// protein binding /// growth factor activity /// protein heterodimerization activity
216918_s_at	11.07	DST	dystonin	---	cytoskeleton organization and biogenesis /// cell cycle arrest /// cell adhesion /// integrin-mediated signaling pathway /// actin cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis /// intermediate filament cytoskeleton organization and biogenesis	actin binding /// integrin binding /// structural molecule activity /// structural constituent of cytoskeleton /// structural constituent of cytoskeleton /// calcium ion binding /// protein binding /// protein binding /// protein C-terminus binding /// actin filament binding
202351_at	10.52	ITGAV	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	Integrin-mediated_cell_adhesion_KEGG	blood vessel development /// cell adhesion /// cell adhesion /// cell-matrix adhesion /// integrin-mediated signaling pathway /// integrin-mediated signaling pathway	receptor activity /// calcium ion binding /// protein binding /// protein binding
225668_s_at	10.45	PHLDB2	pleckstrin homology-like domain, family B, member 2	---	signal transduction /// intracellular signaling cascade /// lipid catabolic process	phospholipase C activity /// signal transducer activity /// hydrolase activity
202973_x_at	10.42	FAM13A1	family with sequence similarity 13, member A1	---	signal transduction	---

220979_s_at	10.07	ST6GALNAC5	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 5	---	protein amino acid glycosylation	sialyltransferase activity /// transferase activity /// transferase activity, transferring glycosyl groups
202975_s_at	9.827	RHOBTB3	Rho-related BTB domain containing 3	---	protein localization	GTPase activity /// protein binding
239288_at	9.624	TNIK	TRAF2 and NCK interacting kinase	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// response to stress /// protein kinase cascade /// JNK cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// small GTPase regulator activity /// ATP binding /// ATP binding /// kinase activity /// transferase activity
201941_at	9.279	CPD	carboxypeptidase D	---	proteolysis	carboxypeptidase activity /// metallocarboxypeptidase activity /// carboxypeptidase A activity /// carboxypeptidase D activity /// carboxypeptidase D activity /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// metallocarboxypeptidase D activity /// hydrolase activity /// metal ion binding
200660_at	9.272	S100A11	S100 calcium binding protein A11	---	signal transduction /// negative regulation of DNA replication /// negative regulation of cell proliferation	calcium ion binding /// protein binding /// protein homodimerization activity /// S100 beta binding /// calcium-dependent protein binding
226897_at	9.209	MARCKS	myristoylated alanine-rich protein kinase C substrate	---	cell motility	actin binding /// calmodulin binding /// calmodulin binding /// actin filament binding
46665_at	9.2	SEMA4C	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	---	multicellular organismal development /// nervous system development /// cell differentiation	receptor activity /// protein binding
217047_s_at	9.114	FAM13A1	family with sequence similarity 13, member A1	---	signal transduction	---
204626_s_at	9.102	ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	Integrin-mediated_cell_adhesion_KEGG	cell-substrate junction assembly /// cell adhesion /// cell adhesion /// cell-matrix adhesion /// integrin-mediated signaling pathway /// multicellular organismal development /// blood coagulation /// regulation of cell migration	receptor activity /// integrin binding /// binding /// protein binding /// protein binding /// identical protein binding
33323_r_at	8.943	SFN	stratifin	Calcium_regulation_in_cardiac_cells /// Smooth_muscle_contraction	regulation of cyclin-dependent protein kinase activity /// release of cytochrome c from mitochondria /// negative regulation of protein kinase activity /// signal transduction /// cell proliferation /// DNA damage response, signal transduction resulting in induction of apoptosis /// apoptotic program /// keratinocyte differentiation /// negative regulation of caspase activity /// skin development	protein binding /// protein kinase C inhibitor activity /// protein domain specific binding
217975_at	8.746	WBP5	WW domain binding protein 5	---	---	protein binding /// WW domain binding
200632_s_at	8.606	NDRG1	N-myc downstream regulated gene 1	---	response to metal ion	protein binding
219674_at	8.001	SLC12A8	solute carrier family 12 (potassium/chloride transporters), member 8	---	transport /// ion transport /// potassium ion transport	symporter activity /// potassium ion binding /// chloride ion binding
241812_at	7.906	LOC26010	viral DNA polymerase-transactivated protein 6	---	---	---
211986_at	7.719	AHNAK	AHNAK nucleoprotein	---	nervous system development	nucleic acid binding /// protein binding
225102_at	7.543	MGLL	monoglyceride lipase	---	lipid metabolic process /// inflammatory response	catalytic activity /// carboxylesterase activity /// lysophospholipase activity /// hydrolase activity /// acylglycerol lipase activity
213262_at	7.204	SACS	spastic ataxia of Charlevoix-Saguenay (sacsin)	---	protein folding /// protein modification process	ATP binding /// heat shock protein binding
201063_at	7.178	RCN1	reticulocalbin 1, EF-hand calcium binding domain	---	---	calcium ion binding /// calcium ion binding
212763_at	7.03	CAMSAP1L1	calmodulin regulated spectrin-associated protein 1-like 1	---	---	---
239973_at	6.676	---	Transcribed locus	---	---	---
33322_i_at	6.439	SFN	stratifin	Calcium_regulation_in_cardiac_cells /// Smooth_muscle_contraction	regulation of cyclin-dependent protein kinase activity /// release of cytochrome c from mitochondria /// negative regulation of protein kinase activity /// signal transduction /// cell proliferation /// DNA damage response, signal transduction resulting in induction of apoptosis /// apoptotic program /// keratinocyte differentiation /// negative regulation of caspase activity /// skin development	protein binding /// protein kinase C inhibitor activity /// protein domain specific binding
200770_s_at	6.433	LAMC1	laminin, gamma 1 (formerly LAMB2)	Inflammatory_Response_Pathway	protein complex assembly /// cell adhesion /// cell adhesion /// endoderm development /// cell migration /// extracellular matrix disassembly /// hemidesmosome assembly /// positive regulation of epithelial cell proliferation	extracellular matrix structural constituent /// protein binding
203662_at	6.339	FEZ1	fasciculation and elongation protein zeta 1 (zygin I)	---	cell adhesion /// nervous system development /// axon guidance	protein binding /// protein binding /// gamma-tubulin binding
212110_at	6.112	SLC39A14	solute carrier family 39 (zinc transporter), member 14	---	transport /// ion transport /// zinc ion transport /// metal ion transport	zinc ion binding /// metal ion transmembrane transporter activity
204341_at	5.993	TRIM16	tripartite motif-containing 16	---	---	transcription factor activity /// zinc ion binding /// metal ion binding
222761_at	5.977	BIVM	basic, immunoglobulin-like variable motif containing	---	---	---
200984_s_at	5.84	CD59	CD59 molecule, complement regulatory protein	---	defense response /// immune response /// cell surface receptor linked signal transduction /// blood coagulation	protein binding
218793_s_at	5.785	SCML1	sex comb on midleg-like 1 (Drosophila)	---	transcription /// regulation of transcription, DNA-dependent /// anatomical structure morphogenesis	DNA binding /// transcription factor activity
204142_at	5.742	ENOSF1	enolase superfamily member 1	---	metabolic process	magnesium ion binding /// catalytic activity /// transferase activity /// isomerase activity
209677_at	5.69	PRKCI	protein kinase C, iota	G_Protein_Signaling /// Wnt_signaling	protein amino acid phosphorylation /// protein amino acid phosphorylation /// protein targeting to membrane /// cytoskeleton organization and biogenesis /// actin filament organization /// signal transduction /// intracellular signaling cascade /// membrane organization and biogenesis /// vesicle-mediated transport /// establishment of apical/basal cell polarity /// eye photoreceptor cell development /// establishment and/or maintenance of epithelial cell polarity /// cell-cell junction assembly and maintenance /// secretion /// Golgi vesicle budding	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein kinase C activity /// atypical protein kinase C activity /// atypical protein kinase C activity /// atypical protein kinase C activity /// protein binding /// ATP binding /// ATP binding /// phospholipid binding /// zinc ion binding /// kinase activity /// transferase activity /// diacylglycerol binding /// metal ion binding
209344_at	5.686	TPM4	tropomyosin 4	Striated_muscle_contraction	cell motility	actin binding /// structural constituent of muscle
211186_s_at	5.634	AAK1	AP2 associated kinase 1	Fatty_Acid_Synthesis	protein amino acid phosphorylation	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// ATP binding /// kinase activity /// transferase activity
209122_at	5.589	ADFP	adipose differentiation-related protein	---	---	---
214077_x_at	5.541	MEIS3P1	Meis homeobox 3 pseudogene 1	---	regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// sequence-specific DNA binding /// sequence-specific DNA binding
225718_at	5.54	KIAA1715	KIAA1715	---	multicellular organismal development	protein binding
209288_s_at	5.414	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	---	signal transduction /// regulation of cell shape	protein binding /// cytoskeletal regulatory protein binding
1566633_at	5.344	---	CDNA: FLJ20875 fis, clone ADKA02835	---	---	---
201562_s_at	5.312	SORD	sorbitol dehydrogenase	---	sorbitol metabolic process /// response to osmotic stress /// visual perception /// metabolic process /// response to hormone stimulus /// response to nutrient levels /// response to drug /// response to cadmium ion /// response to copper ion	catalytic activity /// L-iditol 2-dehydrogenase activity /// binding /// zinc ion binding /// oxidoreductase activity /// identical protein binding /// metal ion binding
224963_at	5.157	SCARB2	scavenger receptor class B, member 2	---	cell adhesion	receptor activity /// protein binding
225847_at	5.113	AADACL1	arylacetamide deacetylase-like 1	---	metabolic process	hydrolase activity
203735_x_at	5.087	PPFIBP1	PTPRF interacting protein, binding protein 1 (liprin beta 1)	---	cell adhesion /// DNA integration	DNA binding /// protein binding /// protein binding /// integrase activity
243444_at	5.001	---	Transcribed locus	---	---	---
229538_s_at	4.982	IQGAP3	IQ motif containing GTPase activating protein 3	---	signal transduction /// small GTPase mediated signal transduction /// regulation of small GTPase mediated signal transduction	GTPase activator activity /// Ras GTPase activator activity /// calmodulin binding
206995_x_at	4.934	SCARF1	scavenger receptor class F, member 1	---	cholesterol catabolic process /// receptor-mediated endocytosis /// cell adhesion	receptor activity /// transmembrane receptor activity /// scavenger receptor activity /// protein binding /// low-density lipoprotein binding
225406_at	4.916	TWSG1	twisted gastrulation homolog 1 (Drosophila)	---	ossification /// mesoderm formation /// multicellular organismal development /// embryonic development /// tissue development /// hemopoiesis /// cell differentiation /// BMP signaling pathway /// positive regulation of BMP signaling pathway /// negative regulation of BMP signaling pathway /// forebrain development /// camera-type eye development /// negative regulation of osteoblast differentiation	protein binding
213503_x_at	4.764	ANXA2	annexin A2	Prostaglandin_synthesis_regulation	skeletal development	phospholipase inhibitor activity /// phospholipase inhibitor activity /// calcium ion binding /// protein binding /// calcium-dependent phospholipid binding /// cytoskeletal protein binding
232300_at	4.712	LOC100128309	hypothetical protein LOC100128309	---	---	---
228913_at	4.673	---	CDNA FLJ30519 fis, clone BRAWH2000869	---	---	---
236249_at	4.642	IKIP	IKK interacting protein	---	induction of apoptosis /// response to X-ray	protein binding

210427_x_at	4.629	ANXA2	annexin A2	Prostaglandin_synthesis_regulation	skeletal development	phospholipase inhibitor activity /// phospholipase inhibitor activity /// calcium ion binding /// protein binding /// calcium-dependent phospholipid binding /// cytoskeletal protein binding
200983_x_at	4.598	CD59	CD59 molecule, complement regulatory protein	---	defense response /// immune response /// cell surface receptor linked signal transduction /// blood coagulation	protein binding
209038_at	4.595	PTPRK	protein tyrosine phosphatase, receptor type, K	---	protein amino acid dephosphorylation /// dephosphorylation	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// receptor activity /// transmembrane receptor protein tyrosine phosphatase activity /// hydrolase activity /// phosphoric monoester hydrolase activity
224893_at	4.518	ATL3	atlastin 3	---	---	nucleotide binding /// GTPase activity /// GTP binding
235766_x_at	4.5	---	CDNA clone IMAGE:5746639	---	---	---
212812_at	4.44	---	CDNA: FLJ22642 fis, clone HSI06970	---	---	---
203837_at	4.39	MAP3K5	mitogen-activated protein kinase kinase kinase 5	---	MAPKKK cascade /// MAPKKK cascade /// protein amino acid phosphorylation /// apoptosis /// response to stress /// activation of JNK activity /// induction of apoptosis by extracellular signals	nucleotide binding /// magnesium ion binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// MAP kinase kinase kinase activity /// MAP kinase kinase kinase activity /// MAP kinase kinase kinase activity /// protein binding /// ATP binding /// ATP binding /// caspase activator activity /// kinase activity /// transferase activity /// identical protein binding /// protein homodimerization activity /// metal ion binding
200931_s_at	4.321	VCL	vinculin	Integrin-mediated_cell_adhesion_KEGG	cell motility /// cell adhesion /// cell adhesion /// lamellipodium biogenesis /// negative regulation of cell migration /// apical junction assembly	actin binding /// actin binding /// structural molecule activity /// protein binding /// protein binding /// protein binding /// oxidoreductase activity /// alpha-catenin binding
227432_s_at	4.316	---	Transcribed locus, strongly similar to NP_058767.1 insulin receptor [Rattus norvegicus]	---	---	---
201690_x_at	4.28	ANXA2	annexin A2	Prostaglandin_synthesis_regulation	skeletal development	phospholipase inhibitor activity /// phospholipase inhibitor activity /// calcium ion binding /// protein binding /// calcium-dependent phospholipid binding /// cytoskeletal protein binding
209311_at	4.266	BCL2L2	BCL2-like 2	Apoptosis_KEGG	apoptosis /// anti-apoptosis /// spermatogenesis /// regulation of apoptosis	protein binding
224973_at	4.255	FAM46A	family with sequence similarity 46, member A	---	---	---
235016_at	4.229	REEP3	receptor accessory protein 3	---	---	---
244428_at	4.144	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	---	DNA methylation /// DNA methylation /// DNA methylation	DNA binding /// DNA binding /// DNA (cytosine-5-)-methyltransferase activity /// DNA (cytosine-5-)-methyltransferase activity /// DNA (cytosine-5-)-methyltransferase activity /// protein binding /// methyltransferase activity /// zinc ion binding /// transferase activity /// metal ion binding
209708_at	4.119	MOXD1	monooxygenase, DBH-like 1	---	histidine catabolic process /// catecholamine metabolic process /// cellular process	catalytic activity /// monooxygenase activity /// dopamine beta-monooxygenase activity /// copper ion binding /// oxidoreductase activity /// metal ion binding
228542_at	4.068	---	Transcribed locus	---	---	---
219362_at	4.061	HERC6	hect domain and RLD 6	---	protein modification process /// ubiquitin cycle	ubiquitin-protein ligase activity /// ligase activity
225112_at	4.042	ABI2	abl interactor 2	---	cell motility /// cytoskeleton organization and biogenesis /// actin polymerization and/or depolymerization /// cell migration /// peptidyl-tyrosine phosphorylation	DNA binding /// SH3/SH2 adaptor activity /// cytoskeletal adaptor activity /// SH3 domain binding /// kinase binding
230737_s_at	4.023	LOC387647	patched domain containing 3 pseudogene	---	---	---
225603_s_at	4.021	LOC286144	hypothetical LOC286144	---	---	---
233877_at	3.999	---	CDNA FLJ20770 fis, clone COL06509	---	---	---
224389_at	3.991	PDCD1LG2	programmed cell death 1 ligand 2	---	immune response	receptor activity
242423_x_at	3.976	---	CDNA clone IMAGE:6576427	---	---	---
202946_s_at	3.956	BTBD3	BTB (POZ) domain containing 3	---	---	protein binding
220676_at	3.82	PGAP1	post-GPI attachment to proteins 1	---	GPI anchor metabolic process /// transport /// intracellular protein transport /// protein transport /// myo-inositol transport	catalytic activity /// nuclease activity /// hydrolase activity /// hydrolase activity, acting on ester bonds /// phosphoric ester hydrolase activity
1553956_at	3.801	ALS2CR4	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 4	---	---	---
225685_at	3.776	---	CDNA FLJ31353 fis, clone MESAN2000264	---	---	---
204165_at	3.775	WASF1	WAS protein family, member 1	---	protein complex assembly /// cell motility /// actin filament polymerization	nucleic acid binding /// actin binding /// helicase activity /// ATP binding /// ATP-dependent helicase activity
235391_at	3.746	FAM92A1	family with sequence similarity 92, member A1	---	---	---
212739_s_at	3.742	NME4	non-metastatic cells 4, protein expressed in	---	GTP biosynthetic process /// UTP biosynthetic process /// CTP biosynthetic process /// nucleoside metabolic process /// nucleotide metabolic process	nucleotide binding /// magnesium ion binding /// nucleoside diphosphate kinase activity /// nucleoside diphosphate kinase activity /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
217608_at	3.65	SFRS12IP1	SFRS12-interacting protein 1	---	nucleosome assembly /// mRNA processing /// RNA splicing	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
212875_s_at	3.622	C2CD2	C2 calcium-dependent domain containing 2	---	---	---
220121_at	3.608	LIN54	lines homolog 1 (Drosophila)	---	---	---
220955_x_at	3.562	RAB23	RAB23, member RAS oncogene family	---	transport /// signal transduction /// small GTPase mediated signal transduction /// nervous system development /// protein transport /// spinal cord dorsal/ventral patterning /// embryonic digit morphogenesis /// negative regulation of proteolysis	nucleotide binding /// GTP binding
244704_at	3.533	NFYB	nuclear transcription factor Y, beta	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	DNA binding /// DNA binding /// transcription factor activity /// protein binding /// protein binding /// sequence-specific DNA binding
203343_at	3.515	UGDH	UDP-glucose dehydrogenase	---	UDP-glucose metabolic process /// glycosaminoglycan biosynthetic process /// UDP-glucuronate biosynthetic process /// metabolic process /// oxidation reduction	catalytic activity /// UDP-glucose 6-dehydrogenase activity /// UDP-glucose 6-dehydrogenase activity /// binding /// electron donor activity /// electron carrier activity /// oxidoreductase activity /// oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor /// coenzyme binding /// NAD binding
217884_at	3.514	KCTD3	potassium channel tetramerisation domain containing 3	---	potassium ion transport	ion channel activity /// voltage-gated potassium channel activity /// protein binding /// protein binding
201341_at	3.366	ENC1	ectodermal-neural cortex (with BTB-like domain)	---	ubiquitin cycle /// multicellular organismal development /// multicellular organismal development /// nervous system development	actin binding /// protein binding
218729_at	3.349	LXN	latexin	---	detection of temperature stimulus involved in sensory perception of pain	enzyme inhibitor activity /// protein binding /// metalloendopeptidase inhibitor activity
214582_at	3.345	PDE3B	phosphodiesterase 3B, cGMP-inhibited	---	signal transduction /// endocrine pancreas development /// glucose homeostasis /// regulation of insulin secretion	catalytic activity /// 3',5'-cyclic-nucleotide phosphodiesterase activity /// cGMP-inhibited cyclic-nucleotide phosphodiesterase activity /// hydrolase activity
241643_at	3.34	TLK1	Tousled-like kinase 1	---	regulation of chromatin assembly or disassembly /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// intracellular protein transport /// response to DNA damage stimulus /// cell cycle /// intracellular signaling cascade /// chromatin modification	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity
203508_at	3.3	TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B	Apoptosis /// Apoptosis_GenMAPP /// Apoptosis_KEGG /// Inflammatory_Response_Pathway	apoptosis	receptor activity /// tumor necrosis factor receptor activity /// protein binding
225221_at	3.297	---	CDNA FLJ32068 fis, clone OCBBF1000114	---	---	---
206302_at	3.233	HMHB1	histocompatibility (minor) HB-1	---	immune response	---
227156_at	3.219	TNRC6	trinucleotide repeat containing 6	---	---	---
1557450_s_at	3.178	---	I.M.A.G.E. clone 241447, mRNA sequence	---	---	---
222778_s_at	3.178	WHSC1	Wolf-Hirschhorn syndrome candidate 1	---	transcription /// regulation of transcription, DNA-dependent /// anatomical structure morphogenesis /// chromatin modification	DNA binding /// protein binding /// methyltransferase activity /// zinc ion binding /// transferase activity /// histone-lysine N-methyltransferase activity /// metal ion binding
223849_s_at	3.167	MOV10	Mov10, Moloney leukemia virus 10, homolog (mouse)	---	multicellular organismal development	nucleotide binding /// helicase activity /// protein binding /// ATP binding /// hydrolase activity
244688_at	3.154	---	Transcribed locus	---	---	---
201641_at	3.136	BST2	bone marrow stromal cell antigen 2	---	humoral immune response /// cell-cell signaling /// multicellular organismal development /// cell proliferation /// B cell activation /// positive regulation of I-kappaB kinase/NF-kappaB cascade	signal transducer activity
201942_s_at	3.132	CPD	carboxypeptidase D	---	proteolysis	carboxypeptidase activity /// metallocarboxypeptidase activity /// carboxypeptidase A activity /// carboxypeptidase D activity /// carboxypeptidase D activity /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// metallocarboxypeptidase D activity /// hydrolase activity /// metal ion binding
222875_at	3.047	DHX33	DEAH (Asp-Glu-Ala-His) box polypeptide 33	---	---	nucleotide binding /// nucleic acid binding /// helicase activity /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity
225880_at	3.045	---	CDNA FLJ11174 fis, clone PLACE1007367	---	---	---

208862_s_at	3.052	CTNND1	catenin (cadherin-associated protein), delta 1	---	transcription /// regulation of transcription, DNA-dependent /// cell adhesion /// cell adhesion /// Wnt receptor signaling pathway /// cell-cell adhesion /// cell redox homeostasis	structural molecule activity /// binding /// protein binding
201661_s_at	2.984	ACSL3	acyl-CoA synthetase long-chain family member 3	Fatty_Acid_Degradation /// Mitochondrial_fatty_acid_betaoxidation	lipid metabolic process /// fatty acid metabolic process /// metabolic process	magnesium ion binding /// catalytic activity /// fatty-acyl-CoA synthase activity /// long-chain-fatty-acid-CoA ligase activity /// protein binding /// ligase activity
200757_s_at	2.975	CALU	calumenin	---	---	calcium ion binding /// calcium ion binding /// calcium ion binding
241600_at	2.808	---	Transcribed locus	---	---	---
1559391_s_at	2.802	---	Partial mRNA; ID: EE2-8E	---	---	---
226183_at	2.726	---	MRNA; cDNA DKFZp686L15210 (from clone DKFZp686L15210)	---	---	---
200796_s_at	2.713	GPX1	glutathione peroxidase 1	---	response to reactive oxygen species /// release of cytochrome c from mitochondria /// glutathione metabolic process /// anti-apoptosis /// response to oxidative stress /// induction of apoptosis by oxidative stress /// UV protection /// response to selenium ion /// regulation of cell redox homeostasis /// regulation of mammary gland epithelial cell proliferation /// regulation of gene expression, epigenetic /// response to hydrogen peroxide /// hydrogen peroxide catabolic process /// hydrogen peroxide catabolic process /// negative regulation of caspase activity /// heart contraction	peroxidase activity /// glutathione peroxidase activity /// glutathione peroxidase activity /// selenium binding /// proteasome inhibitor activity /// oxidoreductase activity /// SH3 domain binding /// glutathione binding
208731_at	2.711	RAB2A	RAB2A, member RAS oncogene family	---	transport /// ER to Golgi vesicle-mediated transport /// small GTPase mediated signal transduction /// protein transport /// vesicle-mediated transport	nucleotide binding /// GTPase activity /// GTP binding
241336_at	2.643	---	Transcribed locus	---	---	---
213275_x_at	2.638	CTSB	cathepsin B	---	proteolysis /// proteolysis /// response to wounding /// regulation of apoptosis /// regulation of catalytic activity	cysteine-type endopeptidase activity /// cathepsin B activity /// cathepsin B activity /// protein binding /// peptidase activity /// cysteine-type peptidase activity /// hydrolase activity /// kininogen binding
216072_at	2.632	---	Transcribed locus	---	---	---
205702_at	2.6	PHTF1	putative homeodomain transcription factor 1	---	transcription /// regulation of transcription, DNA-dependent	DNA binding /// transcription factor activity
1565734_at	2.595	---	CDNA FLJ39816 fis, clone SPLEN2010119	---	---	---
226510_at	2.587	HEATR5A	HEAT repeat containing 5A	---	---	binding
1568813_at	2.571	---	CDNA clone IMAGE:4620359	---	---	---
212845_at	2.546	SAMD4A	sterile alpha motif domain containing 4A	---	positive regulation of translation	translation repressor activity
226040_at	2.52	---	MRNA; cDNA DKFZp762N156 (from clone DKFZp762N156)	---	---	---
224722_at	2.514	MIB1	mindbomb homolog 1 (Drosophila)	---	blood vessel development /// in utero embryonic development /// somitogenesis /// neural tube formation /// heart looping /// ubiquitin cycle /// Notch signaling pathway /// heart development /// negative regulation of neuron differentiation /// positive regulation of endocytosis	protein binding /// zinc ion binding /// ligase activity /// metal ion binding
229333_at	2.472	---	Transcribed locus	---	---	---
243568_at	2.44	---	Transcribed locus	---	---	---
213198_at	2.436	ACVR1B	activin A receptor, type IB	---	G1/S transition of mitotic cell cycle /// in utero embryonic development /// hair follicle development /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// induction of apoptosis /// signal transduction /// transmembrane receptor protein serine/threonine kinase signaling pathway /// embryonic development /// negative regulation of cell growth /// positive regulation of activin receptor signaling pathway /// regulation of transcription /// positive regulation of erythrocyte differentiation	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// transmembrane receptor protein serine/threonine kinase activity /// transmembrane receptor protein serine/threonine kinase activity /// receptor activity /// transforming growth factor beta receptor activity /// protein binding /// ATP binding /// ATP binding /// kinase activity /// activin receptor activity, type I /// transferase activity /// activin receptor activity /// growth factor binding /// manganese ion binding /// SMAD binding /// metal ion binding /// activin binding /// activin binding
238065_at	2.429	TPM3	tropomyosin 3	---	cell motility /// regulation of muscle contraction	actin binding /// protein binding
214012_at	2.412	ERAP1	endoplasmic reticulum aminopeptidase 1	---	angiogenesis /// proteolysis /// membrane protein ectodomain proteolysis /// immune response /// regulation of blood pressure /// regulation of blood pressure /// antigen processing and presentation of endogenous peptide antigen via MHC class I /// antigen processing and presentation of endogenous peptide antigen via MHC class I /// regulation of innate immune response /// fat cell differentiation /// positive regulation of angiogenesis	aminopeptidase activity /// aminopeptidase activity /// leucyl aminopeptidase activity /// membrane alanyl aminopeptidase activity /// methionyl aminopeptidase activity /// methionyl aminopeptidase activity /// receptor activity /// interleukin-6 receptor binding /// interleukin-1, Type II receptor binding /// protein binding /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// zinc ion binding /// hydrolase activity /// metal ion binding
227329_at	2.398	ZBTB46	zinc finger and BTB domain containing 46	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// protein binding /// zinc ion binding /// metal ion binding
223376_s_at	2.373	BRI3	brain protein I3	---	---	---
1557077_a_at	2.36	---	CDNA FLJ32587 fis, clone SPLEN2000402	---	---	---
210137_s_at	2.331	DCTD	dCMP deaminase	---	pyrimidine nucleotide metabolic process /// nucleotide biosynthetic process	catalytic activity /// dCMP deaminase activity /// dCMP deaminase activity /// zinc ion binding /// hydrolase activity /// metal ion binding
230805_at	2.298	---	Transcribed locus, strongly similar to NP_001028866.1 sterol regulatory element binding factor 2 [Rattus norvegicus]	---	---	---
214670_at	2.283	ZKSCAN1	zinc finger with KRAB and SCAN domains 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// transcription factor activity /// zinc ion binding /// metal ion binding
1569093_at	2.274	---	Transcribed locus	---	---	---
232685_at	2.259	---	CDNA: FLJ21564 fis, clone COL06452	---	---	---
213551_x_at	2.238	PCGF2	polycomb group ring finger 2	---	transcription /// regulation of transcription, DNA-dependent	DNA binding /// transcription factor activity /// protein binding /// zinc ion binding /// metal ion binding
233368_at	2.21	FLJ14311	hypothetical gene FLJ14311	---	---	---
224663_s_at	2.209	CFL2	cofilin 2 (muscle)	G13_Signaling_Pathway	---	actin binding /// protein binding
201576_at	2.204	PODXL	podocalyxin-like	---	negative regulation of cell adhesion /// leukocyte migration	protein binding
225876_at	2.182	NIPAL3	NIPA-like domain containing 3	---	---	---
225831_at	2.181	LUZP1	leucine zipper protein 1	---	---	---
213644_at	2.16	CCDC46	coiled-coil domain containing 46	---	---	---
229321_s_at	2.159	---	CDNA FLJ36002 fis, clone OCBBF2011914	---	---	---
200001_at	2.145	CAPNS1	calpain, small subunit 1	Integrin-mediated_cell_adhesion_KEGG	positive regulation of cell proliferation	calcium-dependent cysteine-type endopeptidase activity /// calcium ion binding /// protein binding /// protein binding
227515_x_at	2.136	PROSC	Proline synthetase co-transcribed homolog (bacterial)	---	---	---
218066_at	2.107	SLC12A7	solute carrier family 12 (potassium/chloride transporters), member 7	---	transport /// transport /// ion transport /// potassium ion transport /// sodium ion transport /// chloride transport	transporter activity /// symporter activity /// cation:chloride symporter activity /// potassium:chloride symporter activity /// potassium ion binding
226234_at	2.104	---	CDNA FLJ33772 fis, clone BRSSN2000175	---	---	---
221977_at	2.096	TBX2	T-box 2	---	transcription /// regulation of transcription, DNA-dependent /// multicellular organismal development /// cell aging /// positive regulation of cell proliferation /// regulation of transcription /// negative regulation of transcription, DNA-dependent	DNA binding /// DNA binding /// transcription factor activity /// transcription repressor activity /// sequence-specific DNA binding
1568873_at	2.09	ZNF519	zinc finger protein 519	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
244427_at	2.068	KIF23	Kinesin family member 23	---	mitotic spindle elongation /// microtubule-based movement /// cell cycle /// mitosis /// cell division	nucleotide binding /// motor activity /// microtubule motor activity /// microtubule motor activity /// protein binding /// ATP binding
202225_at	2.066	---	CDNA FLJ38130 fis, clone D6OST2000464	---	---	---
1557963_at	2.054	ZKSCAN1	zinc finger with KRAB and SCAN domains 1	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// transcription factor activity /// zinc ion binding /// metal ion binding
230534_at	2.02	MGC15634	hypothetical protein MGC15634	---	---	---
1569289_at	2.01	BIVM	Basic, immunoglobulin-like variable motif containing	---	---	---
243091_at	2.002	---	Transcribed locus	---	---	---
216316_x_at	2	GK /// GK3P	glycerol kinase /// glycerol kinase 3 pseudogene	---	carbohydrate metabolic process /// glycerol metabolic process /// glycerol metabolic process /// glycerol-3-phosphate metabolic process	nucleotide binding /// glycerol kinase activity /// glycerol kinase activity /// ATP binding /// kinase activity /// transferase activity
228889_at	1.979	C14orf128	chromosome 14 open reading frame 128	---	---	---
1568689_at	1.972	---	Transcribed locus	---	---	---
206863_s_at	1.956	MAP3K7	Mitogen-activated protein kinase kinase kinase 7	---	positive regulation of T cell cytokine production /// protein amino acid phosphorylation /// transforming growth factor beta receptor signaling pathway /// activation of NF-kappaB-inducing kinase activity /// positive regulation of interleukin-2 production /// T cell receptor signaling pathway /// positive regulation of T cell activation	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// MAP kinase kinase kinase activity /// MAP kinase kinase kinase activity /// protein tyrosine kinase activity /// protein binding /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
236246_x_at	1.936	LOC663160	Hypothetical protein LOC663160	---	G-protein coupled receptor protein signaling pathway	purinergic nucleotide receptor activity, G-protein coupled
224771_at	1.926	NAV1	neuron navigator 1	---	DNA methylation /// multicellular organismal development /// nervous system development /// cell differentiation	nucleotide binding /// DNA binding /// nucleoside-triphosphatase activity

1554686_at	1.907	STAU2	staufen, RNA binding protein, homolog 2 (Drosophila)	---	transport	RNA binding /// double-stranded RNA binding /// double-stranded RNA binding
226985_at	1.887	PRKAA1	protein kinase, AMP-activated, alpha 1 catalytic subunit	Fatty_Acid_Synthesis	activation of MAPK activity /// response to hypoxia /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// fatty acid biosynthetic process /// steroid biosynthetic process /// cholesterol biosynthetic process /// signal transduction /// lipid biosynthetic process /// sterol biosynthetic process /// positive regulation of cholesterol biosynthetic process /// positive regulation of anti-apoptosis /// negative regulation of glucosylceramide biosynthetic process	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// cAMP-dependent protein kinase activity /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
219031_s_at	1.858	NIP7	nuclear import 7 homolog (S. cerevisiae)	---	ribosome biogenesis and assembly /// ribosome assembly	RNA binding /// protein binding /// protein binding
231810_at	1.819	BRI3BP	BRI3 binding protein	---	---	---
221877_at	1.802	---	CDNA FLJ38849 fis, clone MESAN2008996	---	---	---
216478_at	1.766	LOC100133303	hypothetical protein LOC100133303	---	---	---
203598_s_at	1.754	WBP4	WW domain binding protein 4 (formin binding protein 21)	---	mRNA processing /// RNA splicing	nucleic acid binding /// protein binding /// zinc ion binding /// metal ion binding
219163_at	1.736	ZNF562	zinc finger protein 562	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
220008_at	1.734	SGK269	NKF3 kinase family member	---	protein amino acid phosphorylation	nucleotide binding /// protein kinase activity /// protein tyrosine kinase activity /// non-membrane spanning protein tyrosine kinase activity /// ATP binding /// kinase activity /// transferase activity
218735_s_at	1.733	ZNF544	zinc finger protein 544	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
244154_at	1.713	---	CDNA FLJ34209 fis, clone FCBBF3020699	---	---	---
1557780_at	1.707	---	MRNA; cDNA DKFZp564C1072 (from clone DKFZp564C1072)	---	---	---
233085_s_at	1.686	OBFC2A	oligonucleotide/oligosaccharide-binding fold containing 2A	---	---	nucleic acid binding
228177_at	1.648	CREBBP	CREB binding protein (Rubinstein-Taybi syndrome)	TGF_Beta_Signaling_Pathway	response to hypoxia /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// protein complex assembly /// signal transduction /// signal transduction /// histone acetylation /// histone acetylation /// N-terminal peptidyl-lysine acetylation /// homeostatic process /// regulation of transcription /// regulation of transcription /// positive regulation of transcription /// positive regulation of transcription	transcription factor activity /// transcription cofactor activity /// transcription coactivator activity /// transcription coactivator activity /// transcription coactivator activity /// transcription coactivator activity /// histone acetyltransferase activity /// histone acetyltransferase activity /// signal transducer activity /// protein binding /// protein binding /// transcription factor binding /// zinc ion binding /// acetyltransferase activity /// transferase activity /// metal ion binding /// MyoD binding
213221_s_at	1.644	SNF1LK2	SNF1-like kinase 2	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// protein kinase cascade /// protein kinase cascade /// insulin receptor signaling pathway /// regulation of insulin receptor signaling pathway	nucleotide binding /// magnesium ion binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
216750_at	1.59	APBB2	amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)	---	cell cycle arrest /// intracellular signaling cascade /// negative regulation of cell growth /// regulation of transcription /// negative regulation of S phase of mitotic cell cycle	beta-amyloid binding /// protein binding /// protein binding /// protein binding /// transcription factor binding
226080_at	1.583	MYO1C	myosin IC	---	---	nucleotide binding /// motor activity /// actin binding /// protein binding /// calmodulin binding /// ATP binding
234844_at	1.463	ZNF407	zinc finger protein 407	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
228722_at	1.447	PRMT2	protein arginine methyltransferase 2	---	protein amino acid methylation /// signal transduction	signal transducer activity /// protein binding /// methyltransferase activity /// transferase activity /// identical protein binding
222614_at	1.362	RWDD2B	RWD domain containing 2B	---	---	---
211089_s_at	1.31	NEK3	NIMA (never in mitosis gene a)-related kinase 3	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// cell cycle /// cell cycle /// mitosis /// mitosis /// cell division	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein tyrosine kinase activity /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity /// metal ion binding
215268_at	1.267	KIAA0754	hypothetical LOC643314	---	---	---

GENES DOWNREGULATED EXCLUSIVELY IN MEMORY B CELLS UPON TRANSFORMATION BY EBV

Probe Set ID	Fold Change	Gene Symbol	Gene Title	Pathway	go biological process term	go molecular function term
243968_x_at	-94.08	FCRL1	Fc receptor-like 1	---	---	receptor activity
231455_at	-91.22	FLJ42418	FLJ42418 protein	---	---	---
205997_at	-84.7	ADAM28	ADAM metallopeptidase domain 28	---	proteolysis /// spermatogenesis	metalloendopeptidase activity /// peptidase activity /// metallopeptidase activity /// metallopeptidase activity /// zinc ion binding /// hydrolase activity /// metal ion binding
209189_at	-63.73	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	Smooth_muscle_contraction /// TGF_Beta_Signaling_Pathway	DNA methylation /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// inflammatory response /// nervous system development /// regulation of transcription	DNA binding /// double-stranded DNA binding /// transcription factor activity /// specific RNA polymerase II transcription factor activity /// protein binding /// sequence-specific DNA binding /// protein heterodimerization activity /// protein dimerization activity
208092_s_at	-46.91	FAM49A	family with sequence similarity 49, member A	---	---	---
240923_at	-34.6	---	Transcribed locus	---	---	---
219655_at	-31.21	C7orf10	chromosome 7 open reading frame 10	---	metabolic process	catalytic activity /// transferase activity
216615_s_at	-30.78	HTR3A	5-hydroxytryptamine (serotonin) receptor 3A	---	transport /// transport /// transport /// ion transport /// synaptic transmission /// digestion	receptor activity /// receptor activity /// serotonin receptor activity /// serotonin receptor activity /// ion channel activity /// extracellular ligand-gated ion channel activity /// extracellular ligand-gated ion channel activity /// serotonin-activated cation-selective channel activity /// serotonin-activated cation-selective channel activity /// neurotransmitter receptor activity
226646_at	-25.86	KLF2	Kruppel-like factor 2 (lung)	---	transcription /// regulation of transcription, DNA-dependent /// positive regulation of transcription	nucleic acid binding /// DNA binding /// transcription factor activity /// protein binding /// zinc ion binding /// transcription activator activity /// transcription regulator activity /// metal ion binding
1556451_at	-24.35	---	MRNA; cDNA DKFZp667B1520 (from clone DKFZp667B1520)	---	---	---
203641_s_at	-23.58	COBLL1	COBL-like 1	---	---	---
235739_at	-21.49	---	Transcribed locus	---	---	---
219517_at	-19.3	ELL3	elongation factor RNA polymerase II-like 3	---	transcription /// RNA elongation /// regulation of transcription, DNA-dependent /// RNA elongation from RNA polymerase II promoter /// spermatogenesis /// positive regulation of transcription from RNA polymerase II promoter, global	translation elongation factor activity /// protein binding /// positive transcription elongation factor activity /// RNA polymerase II transcription elongation factor activity
236353_at	-18.72	KIAA0746	KIAA0746 protein	---	---	binding
222378_at	-16.82	---	Transcribed locus	---	---	---
236338_at	-15.57	---	---	---	---	---
215933_s_at	-15.19	HHEX	hematopoietically expressed homeobox	---	liver development /// transcription /// regulation of transcription, DNA-dependent /// mRNA export from nucleus /// cell cycle /// multicellular organismal development /// cell proliferation /// anterior/posterior pattern formation /// positive regulation of specific transcription from RNA polymerase II promoter /// negative regulation of specific transcription from RNA polymerase II promoter /// Wnt receptor signaling pathway /// negative regulation of angiogenesis /// cell differentiation /// cell differentiation /// positive regulation of Wnt receptor signaling pathway /// thyroid gland development /// forebrain development /// negative regulation of vascular endothelial growth factor receptor signaling pathway /// embryonic heart tube development /// regulation of cell proliferation /// regulation of transcription	DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// protein binding /// transcription factor binding /// eukaryotic initiation factor 4E binding /// transcription repressor activity /// transcription repressor activity /// sequence-specific DNA binding /// sequence-specific DNA binding /// sequence-specific DNA binding
1566734_at	-14.96	LOC283454	hypothetical protein LOC283454	---	---	---
215330_at	-13.8	---	MRNA; cDNA DKFZp564G222 (from clone DKFZp564G222)	---	---	---
239249_at	-13.04	---	Transcribed locus	---	---	---
216657_x_at	-12.51	IGHA1 /// IGHG1 /// IGHG3 /// IGHM /// IGHV4-31 /// IGHV@ /// LOC100133739	immunoglobulin heavy constant alpha 1 /// immunoglobulin heavy constant gamma 1 (G1m marker) /// immunoglobulin heavy constant gamma 3 (G3m marker) /// immunoglobulin heavy constant mu /// immunoglobulin heavy variable group /// immunoglobulin heavy variable 4-31 /// similar to hCG2038920	---	activation of MAPK activity /// humoral immune response mediated by circulating immunoglobulin /// immune response /// antigen processing and presentation /// positive regulation of B cell proliferation /// early endosome to late endosome transport /// positive regulation of endocytosis /// positive regulation of peptidyl-tyrosine phosphorylation /// B cell receptor signaling pathway	antigen binding /// antigen binding /// antigen binding /// receptor activity /// transmembrane receptor activity /// copper ion binding /// protein binding /// protein binding /// electron carrier activity
232779_at	-12.21	---	CDNA FLJ20781 fis, clone COL04235	---	---	---
228193_s_at	-11.6	C13orf15	Chromosome 13 open reading frame 15	---	regulation of cyclin-dependent protein kinase activity /// cell cycle	protein binding
207761_s_at	-11.3	METTL7A	methyltransferase like 7A	---	metabolic process	methyltransferase activity /// transferase activity
234050_at	-11.13	TAGAP	T-cell activation RhoGTPase activating protein	---	signal transduction	guanyl-nucleotide exchange factor activity
1564248_at	-10.75	LOC100130544	hypothetical protein LOC100130544	---	---	---
244535_at	-10.5	---	Transcribed locus	---	---	---
201041_s_at	-10.4	DUSP1	dual specificity phosphatase 1	---	protein amino acid dephosphorylation /// response to stress /// response to oxidative stress /// cell cycle /// intracellular signaling cascade /// dephosphorylation	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// non-membrane spanning protein tyrosine phosphatase activity /// protein binding /// protein tyrosine/serine/threonine phosphatase activity /// hydrolase activity /// phosphoric monoester hydrolase activity /// MAP kinase tyrosine/serine/threonine phosphatase activity
1553869_at	-10.19	SESN3	sestrin 3	---	cell cycle arrest	---
219396_s_at	-10.12	NEIL1	nei endonuclease VIII-like 1 (E. coli)	---	DNA repair /// base-excision repair /// nucleotide-excision repair /// response to DNA damage stimulus /// metabolic process	nucleic acid binding /// DNA binding /// damaged DNA binding /// catalytic activity /// DNA-(apurinic or apyrimidinic site) lyase activity /// zinc ion binding /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds /// hydrolase activity, hydrolyzing N-glycosyl compounds /// lyase activity
239696_at	-10.02	---	Transcribed locus	---	---	---
235694_s_at	-9.969	SESN3	sestrin 3	---	cell cycle arrest	---
241849_at	-9.87	---	Transcribed locus	---	---	---
212311_at	-9.759	KIAA0746 /// SERINC2	KIAA0746 protein /// serine incorporator 2	---	phosphatidylserine metabolic process /// phosphatidylserine metabolic process /// positive regulation of transferase activity /// positive regulation of transferase activity	binding
217384_x_at	-9.582	LOC100132941 /// LOC647187 /// LOC647224	hypothetical LOC647187 /// hypothetical LOC647224 /// hypothetical protein LOC100132941	---	---	---
226578_s_at	-9.528	LOC100129026	hypothetical protein LOC100129026	---	---	---
1553935_at	-9.509	MGC2848	hypothetical protein MGC2848	---	---	---
217320_at	-9.069	---	Rheumatoid factor RF-ET12	---	---	---
207339_s_at	-8.921	LTB	lymphotoxin beta (TNF superfamily, member 3)	---	immune response /// signal transduction /// cell-cell signaling /// positive regulation of interleukin-12 biosynthetic process	receptor binding /// cytokine activity /// tumor necrosis factor receptor binding
218723_s_at	-8.67	C13orf15	chromosome 13 open reading frame 15	---	regulation of cyclin-dependent protein kinase activity /// cell cycle	protein binding
1561844_at	-8.529	---	MRNA; cDNA DKFZp667G1510 (from clone DKFZp667G1510)	---	---	---
230232_at	-8.434	KIAA0746	KIAA0746 protein	---	---	binding
1559274_at	-8.187	---	CDNA FLJ40940 fis, clone UTERU2008130	---	---	---
225540_at	-8.186	MAP2	microtubule-associated protein 2	MAPK_Cascade	negative regulation of microtubule depolymerization	structural molecule activity /// calmodulin binding
203795_s_at	-7.999	BCL7A	B-cell CLL/lymphoma 7A	---	negative regulation of transcription	---
1554941_at	-7.826	KLHL14	kelch-like 14 (Drosophila)	---	---	protein binding

266_s_at	-7.72	CD24	CD24 molecule	---	response to hypoxia /// cell activation /// regulation of cytokine and chemokine mediated signaling pathway /// regulation of cytokine and chemokine mediated signaling pathway /// response to molecule of bacterial origin /// response to molecule of bacterial origin /// immune response-regulating cell surface receptor signaling pathway /// elevation of cytosolic calcium ion concentration /// neuromuscular synaptic transmission /// induction of apoptosis by intracellular signals /// Wnt receptor signaling pathway /// cell-cell adhesion /// cell migration /// cell migration /// regulation of epithelial cell differentiation /// T cell costimulation /// B cell receptor transport into membrane raft /// chemokine receptor transport out of membrane raft /// negative regulation of transforming growth factor-beta3 production /// positive regulation of activated T cell proliferation /// regulation of phosphorylation /// cholesterol homeostasis /// cholesterol homeostasis /// positive regulation of MAP kinase activity /// regulation of MAPKKK cascade /// response to estrogen stimulus /// respiratory burst ///	signal transducer activity /// protein binding /// protein binding /// protein kinase binding /// carbohydrate binding /// protein tyrosine kinase activator activity
243185_at	-7.439	---	CDNA FLJ42169 fis, clone THYMU2028978	---	---	---
241015_at	-7.356	---	Transcribed locus	---	---	---
1555779_a_at	-7.252	CD79A	CD79a molecule, immunoglobulin-associated alpha	---	immune response /// cell surface receptor linked signal transduction /// B cell differentiation /// B cell differentiation /// B cell proliferation /// B cell proliferation /// B cell activation /// B cell receptor signaling pathway /// B cell receptor signaling pathway	transmembrane receptor activity /// protein binding /// protein binding
232715_at	-7.048	---	CDNA FLJ11544 fis, clone HEMBA1002826	---	---	---
204689_at	-7.041	HHEX	hematopoietically expressed homeobox	---	liver development /// transcription /// regulation of transcription, DNA-dependent /// mRNA export from nucleus /// cell cycle /// multicellular organismal development /// cell proliferation /// anterior/posterior pattern formation /// positive regulation of specific transcription from RNA polymerase II promoter /// negative regulation of specific transcription from RNA polymerase II promoter /// Wnt receptor signaling pathway /// negative regulation of angiogenesis /// cell differentiation /// cell differentiation /// positive regulation of Wnt receptor signaling pathway /// thyroid gland development /// forebrain development /// negative regulation of vascular endothelial growth factor receptor signaling pathway /// embryonic heart tube development /// regulation of cell proliferation /// regulation of transcription	DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// protein binding /// transcription factor binding /// eukaryotic initiation factor 4E binding /// transcription repressor activity /// transcription repressor activity /// sequence-specific DNA binding /// sequence-specific DNA binding /// sequence-specific DNA binding
243967_at	-6.943	AFF3	AF4/FMR2 family, member 3	---	transcription /// regulation of transcription, DNA-dependent /// multicellular organismal development	DNA binding
213281_at	-6.863	JUN	Jun oncogene	Apoptosis /// Apoptosis_GenMAPP /// MAPK_Cascade /// Smooth_muscle_contraction /// TGF_Beta_Signaling_Pathway /// Wnt_signaling	transcription /// regulation of transcription, DNA-dependent /// cellular process /// negative regulation of protein amino acid autophosphorylation /// leading edge cell differentiation /// regulation of transcription /// positive regulation of transcription from RNA polymerase II promoter	DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// RNA polymerase II transcription factor activity /// protein binding /// sequence-specific DNA binding /// protein dimerization activity
243467_at	-6.784	---	CDNA FLJ46553 fis, clone THYMU3038879	---	---	---
230004_at	-6.552	USP24	ubiquitin specific peptidase 24	---	ubiquitin-dependent protein catabolic process /// ubiquitin cycle	ubiquitin thiolesterase activity /// peptidase activity /// cysteine-type peptidase activity /// hydrolase activity
216755_at	-6.544	OSBPL10	oxysterol binding protein-like 10	---	transport /// lipid transport /// steroid metabolic process	---
236545_at	-6.475	---	Transcribed locus	---	---	---
215621_s_at	-6.2	IGHD	immunoglobulin heavy constant delta	---	immune response	antigen binding /// antigen binding
241501_at	-6.158	---	Transcribed locus	---	---	---
242952_at	-6.141	---	---	---	---	---
203796_s_at	-6.006	BCL7A	B-cell CLL/lymphoma 7A	---	negative regulation of transcription	---
216641_x_at	-5.996	IGHA1 /// IGHG1 /// LOC100133862	immunoglobulin heavy constant alpha 1 /// immunoglobulin heavy constant gamma 1 (G1m marker) /// similar to hCG1773549	---	activation of MAPK activity /// humoral immune response mediated by circulating immunoglobulin /// immune response /// antigen processing and presentation /// positive regulation of B cell proliferation /// early endosome to late endosome transport /// positive regulation of endocytosis /// positive regulation of peptidyl-tyrosine phosphorylation /// B cell receptor signaling pathway	antigen binding /// antigen binding /// antigen binding /// receptor activity /// transmembrane receptor activity /// copper ion binding /// protein binding /// protein binding /// electron carrier activity
224339_s_at	-5.837	ANGPTL1	angiopoietin-like 1	---	signal transduction	receptor binding /// receptor binding
239975_at	-5.783	HLA-DPB2	major histocompatibility complex, class II, DP beta 2 (pseudogene)	---	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II /// immune response /// antigen processing and presentation	---
217260_x_at	-5.762	IGHG1	Immunoglobulin heavy constant mu	---	activation of MAPK activity /// humoral immune response mediated by circulating immunoglobulin /// immune response /// antigen processing and presentation /// positive regulation of B cell proliferation /// early endosome to late endosome transport /// positive regulation of endocytosis /// positive regulation of peptidyl-tyrosine phosphorylation /// B cell receptor signaling pathway	antigen binding /// antigen binding /// antigen binding /// receptor activity /// transmembrane receptor activity /// copper ion binding /// protein binding /// protein binding /// electron carrier activity
241879_at	-5.745	---	Transcribed locus	---	---	---
239183_at	-5.681	ANGPTL1	angiopoietin-like 1	---	signal transduction	receptor binding /// receptor binding
243546_at	-5.482	---	Transcribed locus	---	---	---
224496_s_at	-5.365	TMEM107	transmembrane protein 107	---	---	---
241843_at	-5.355	SNORA28	small nucleolar RNA, H/ACA box 28	---	---	---
221841_s_at	-5.339	KLF4	Kruppel-like factor 4 (gut)	---	transcription /// regulation of transcription, DNA-dependent /// mesodermal cell fate determination /// negative regulation of cell proliferation /// negative regulation of transcription, DNA-dependent /// negative regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// transcription factor activity /// zinc ion binding /// zinc ion binding /// transcription activator activity /// transcription repressor activity /// transcription repressor activity /// metal ion binding
217281_x_at	-5.324	IGH@ /// IGHA1 /// IGHA2 /// IGHG1 /// IGHG2 /// IGHG3 /// IGHM /// IGHV4-31 /// LOC100126583 /// LOC100133739 /// LOC652494	immunoglobulin heavy locus /// immunoglobulin heavy constant alpha 1 /// immunoglobulin heavy constant alpha 2 (A2m marker) /// immunoglobulin heavy constant gamma 1 (G1m marker) /// immunoglobulin heavy constant gamma 2 (G2m marker) /// immunoglobulin heavy constant gamma 3 (G3m marker) /// immunoglobulin heavy constant mu /// immunoglobulin heavy variable 4-31 /// similar to Ig heavy chain V-III region VH26 precursor /// hypothetical LOC100126583 /// similar to hCG2038920	---	activation of MAPK activity /// humoral immune response mediated by circulating immunoglobulin /// immune response /// antigen processing and presentation /// positive regulation of B cell proliferation /// early endosome to late endosome transport /// positive regulation of endocytosis /// positive regulation of peptidyl-tyrosine phosphorylation /// B cell receptor signaling pathway	antigen binding /// antigen binding /// antigen binding /// receptor activity /// transmembrane receptor activity /// copper ion binding /// protein binding /// protein binding /// electron carrier activity
215872_at	-5.296	---	---	---	---	---

241619_at	-5.224	CALM1 /// CALM2 /// CALM3	calmodulin 1 (phosphorylase kinase, delta) /// calmodulin 2 (phosphorylase kinase, delta) /// calmodulin 3 (phosphorylase kinase, delta)	Calcium_regulation_in_cardiac_cells /// Calcium_regulation_in_cardiac_cells /// Calcium_regulation_in_cardiac_cells /// G13_Signaling_Pathway /// G13_Signaling_Pathway /// G13_Signaling_Pathway /// Glycogen_Metabolism /// G_Protein_Signaling /// G_Protein_Signaling /// G_Protein_Signaling /// Smooth_muscle_contraction /// Smooth_muscle_contraction /// Smooth_muscle_contraction	G-protein coupled receptor protein signaling pathway	calcium ion binding /// calcium ion binding /// protein binding /// protein binding /// protein binding /// protein binding /// kinase activity /// N-terminal myristoylation domain binding
203761_at	-5.146	SLA	Src-like-adaptor	---	---	SH3/SH2 adaptor activity /// protein binding
238710_at	-5.103	TMEM86A	transmembrane protein 86A	---	---	---
217362_x_at	-5.101	HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene)	---	---	---
237994_at	-4.957	---	Transcribed locus	---	---	---
233817_at	-4.95	---	CDNA FLJ12204 fis, clone MAMMA1000921	---	---	---
219761_at	-4.949	CLEC1A	C-type lectin domain family 1, member A	---	defense response /// cell surface receptor linked signal transduction	transmembrane receptor activity /// binding /// sugar binding
204414_at	-4.925	LARGE	like-glycosyltransferase	---	N-acetylglucosamine metabolic process /// protein amino acid glycosylation /// glycosphingolipid biosynthetic process /// glycoprotein biosynthetic process /// carbohydrate biosynthetic process /// muscle maintenance /// muscle maintenance	acetylglucosaminyltransferase activity /// transferase activity /// transferase activity, transferring glycosyl groups /// transferase activity, transferring glycosyl groups /// transferase activity, transferring hexosyl groups
243433_at	-4.889	---	Transcribed locus	---	---	---
235019_at	-4.809	CPM	carboxypeptidase M	---	proteolysis /// anatomical structure morphogenesis	carboxypeptidase activity /// carboxypeptidase activity /// metallocarboxypeptidase activity /// carboxypeptidase A activity /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// hydrolase activity /// metal ion binding
227478_at	-4.803	SETBP1	SET binding protein 1	---	---	DNA binding /// protein binding
1570115_at	-4.777	LOC100134745 /// PP12708	hypothetical LOC100130609 /// hypothetical protein LOC100134745	---	---	---
236780_at	-4.763	PRKACB	protein kinase, cAMP-dependent, catalytic, beta	Calcium_regulation_in_cardiac_cells /// G_Protein_Signaling /// Smooth_muscle_contraction	protein amino acid phosphorylation /// protein amino acid phosphorylation /// signal transduction /// G-protein signaling, coupled to cAMP nucleotide second messenger	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// cAMP-dependent protein kinase activity /// cAMP-dependent protein kinase activity /// cAMP-dependent protein kinase activity /// ATP binding /// ATP binding /// kinase activity /// transferase activity
210369_at	-4.753	SWAP70	SWAP-70 protein	---	somatic cell DNA recombination /// isotype switching	DNA binding /// protein binding /// ATP binding
225177_at	-4.723	RAB11FIP1	RAB11 family interacting protein 1 (class I)	---	transport /// protein transport	protein binding
208383_s_at	-4.687	PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)	Glycolysis_and_Gluconeogenesis	glucose metabolic process /// gluconeogenesis /// lipid metabolic process /// response to insulin stimulus /// glucose homeostasis /// glycerol biosynthetic process from pyruvate /// glycerol biosynthetic process from pyruvate	nucleotide binding /// magnesium ion binding /// phosphoenolpyruvate carboxykinase activity /// phosphoenolpyruvate carboxykinase (GTP) activity /// phosphoenolpyruvate carboxykinase (GTP) activity /// GTP binding /// kinase activity /// lyase activity /// carboxy-lyase activity /// purine nucleotide binding /// manganese ion binding /// carboxylic acid binding
210715_s_at	-4.652	LOC100130414 /// SPINT2	serine peptidase inhibitor, Kunitz type, 2 /// hypothetical protein LOC100130414	---	cell motility	endopeptidase inhibitor activity /// endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity
234140_s_at	-4.625	STIM2	stromal interaction molecule 2	---	transport /// ion transport /// calcium ion transport /// negative regulation of calcium ion transport via store-operated calcium channel	calcium ion binding /// protein binding
242836_at	-4.565	---	Transcribed locus	---	---	---
222519_s_at	-4.525	IFT57	intraflagellar transport 57 homolog (Chlamydomonas)	---	transcription /// regulation of transcription, DNA-dependent /// apoptosis /// apoptosis /// caspase activation /// regulation of apoptosis	DNA binding /// protein binding /// protein binding
215750_at	-4.514	KIAA1659	KIAA1659 protein	---	---	---
239835_at	-4.442	KBTBD8	kelch repeat and BTB (POZ) domain containing 8	---	---	protein binding
206036_at	-4.366	REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)	---	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// positive regulation of I-kappaB kinase/NF-kappaB cascade /// regulation of transcription	DNA binding /// transcription factor activity /// transcription factor activity /// signal transducer activity /// protein binding
233251_at	-4.298	STRBP	Chromosome 9 open reading frame 45	---	multicellular organismal development /// spermatogenesis /// cell differentiation	DNA binding /// RNA binding /// double-stranded RNA binding
219954_s_at	-4.294	GBA3	glucosidase, beta, acid 3 (cytosolic)	---	carbohydrate metabolic process /// metabolic process /// glycoside catabolic process	catalytic activity /// hydrolase activity, hydrolyzing O-glycosyl compounds /// beta-galactosidase activity /// beta-glucosidase activity /// glucosidase activity /// hydrolase activity /// hydrolase activity, acting on glycosyl bonds /// cation binding
201464_x_at	-4.256	JUN	jun oncogene	Apoptosis /// Apoptosis_GenMAPP /// MAPK_Cascade /// Smooth_muscle_contraction /// TGF_Beta_Signaling_Pathway /// Wnt_signaling	transcription /// regulation of transcription, DNA-dependent /// cellular process /// negative regulation of protein amino acid autophosphorylation /// leading edge cell differentiation /// regulation of transcription /// positive regulation of transcription from RNA polymerase II promoter	DNA binding /// DNA binding /// transcription factor activity /// transcription factor activity /// RNA polymerase II transcription factor activity /// protein binding /// sequence-specific DNA binding /// protein dimerization activity
1553706_at	-4.217	HTRA4	HtrA serine peptidase 4	---	proteolysis	catalytic activity /// serine-type endopeptidase activity /// protein binding /// peptidase activity /// hydrolase activity
1554113_a_at	-4.201	SLC4A8	solute carrier family 4, sodium bicarbonate cotransporter, member 8	---	transport /// ion transport /// sodium ion transport /// anion transport	transporter activity /// inorganic anion exchanger activity /// anion transmembrane transporter activity /// antiporter activity /// anion exchanger activity /// sodium ion binding
1566785_x_at	-4.172	LOC728806	Similar to Vesicle-fusing ATPase (Vesicular-fusion protein NSF) (N-ethylmaleimide sensitive fusion protein) (NEM-sensitive fusion protein)	---	---	nucleotide binding /// ATP binding /// nucleoside-triphosphatase activity
235206_at	-4.15	SCAND1	SCAN domain containing 1	---	regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// induction of apoptosis /// granulocyte differentiation /// regulation of cell proliferation	DNA binding /// transcription factor activity /// transcription factor activity /// transcription factor activity /// transcription coactivator activity /// RNA binding /// protein binding /// protein binding /// transcription factor binding /// identical protein binding /// identical protein binding
228655_at	-4.127	---	Unknown mRNA sequence	---	---	---
217535_at	-4.113	FAM49B	Family with sequence similarity 49, member B	---	---	---
242499_at	-4.098	U2AF1	U2 small nuclear RNA auxiliary factor 1	mRNA_processing_Reactome	nuclear mRNA splicing, via spliceosome /// mRNA processing /// mRNA processing /// RNA splicing /// RNA splicing	nucleotide binding /// nucleic acid binding /// RNA binding /// protein binding /// zinc ion binding /// metal ion binding
235851_s_at	-4.094	GNAS	GNAS complex locus	---	signal transduction /// signal transduction /// G-protein coupled receptor protein signaling pathway /// G-protein signaling, adenylate cyclase activating pathway /// G-protein signaling, adenylate cyclase activating pathway /// activation of adenylate cyclase activity /// female pregnancy /// sensory perception of smell /// protein secretion /// intracellular transport	nucleotide binding /// GTPase activity /// GTPase activity /// signal transducer activity /// protein binding /// GTP binding /// GTP binding /// guanyl nucleotide binding /// mu-type opioid receptor binding /// identical protein binding
231775_at	-4.087	TNFRSF10A	tumor necrosis factor receptor superfamily, member 10a	---	apoptosis /// induction of apoptosis /// caspase activation /// signal transduction /// signal transduction /// activation of NF-kappaB-inducing kinase activity /// induction of apoptosis via death domain receptors /// induction of apoptosis via death domain receptors	receptor activity /// receptor activity /// death receptor activity /// protein binding /// caspase activator activity /// TRAIL binding
237626_at	-4.07	---	Transcribed locus	---	---	---

1562439_at	-4.066	NCOA3	Nuclear receptor coactivator 3	---	transcription /// transcription /// regulation of transcription, DNA-dependent /// signal transduction /// androgen receptor signaling pathway /// regulation of transcription /// positive regulation of transcription, DNA-dependent	transcription coactivator activity /// transcription coactivator activity /// histone acetyltransferase activity /// histone acetyltransferase activity /// signal transducer activity /// receptor activity /// protein binding /// acyltransferase activity /// transferase activity /// transcription regulator activity /// nuclear hormone receptor binding /// thyroid hormone receptor binding /// protein N-terminus binding /// androgen receptor binding
236688_at	-4.066	FRMPD3	FERM and PDZ domain containing 3	---	---	protein binding
239555_at	-4.056	---	CDNA clone IMAGE:40114646	---	---	---
219313_at	-4.002	GRAMD1C	GRAM domain containing 1C	---	---	---
235193_at	-3.939	---	Transcribed locus	---	---	---
206833_s_at	-3.911	ACYP2	acylphosphatase 2, muscle type	---	phosphate metabolic process	acylphosphatase activity /// acylphosphatase activity /// hydrolase activity
241286_at	-3.884	---	Transcribed locus	---	---	---
224026_at	-3.878	---	---	---	---	---
216979_at	-3.776	NR4A3	nuclear receptor subfamily 4, group A, member 3	Hypertrophy_model	transcription /// regulation of transcription, DNA-dependent	DNA binding /// DNA binding /// transcription factor activity /// steroid hormone receptor activity /// steroid hormone receptor activity /// receptor activity /// ligand-dependent nuclear receptor activity /// thyroid hormone receptor activity /// binding /// zinc ion binding /// sequence-specific DNA binding /// metal ion binding
1556983_a_at	-3.724	---	Full length insert cDNA YW02F03	---	---	---
241461_at	-3.656	---	Transcribed locus	---	---	---
227173_s_at	-3.651	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// protein binding /// sequence-specific DNA binding /// protein dimerization activity
209305_s_at	-3.611	GADD45B	growth arrest and DNA-damage-inducible, beta	---	activation of MAPKKK activity /// activation of MAPKK activity /// negative regulation of protein kinase activity /// apoptosis /// apoptosis /// response to stress /// multicellular organismal development /// cell differentiation	protein binding
228111_s_at	-3.6	DNAH1	dynein, axonemal, heavy chain 1	---	ciliary or flagellar motility /// microtubule-based movement	nucleotide binding /// motor activity /// microtubule motor activity /// microtubule motor activity /// ATP binding /// ATPase activity
229264_at	-3.58	LOC100132999	hypothetical protein LOC100132999	---	---	---
236090_at	-3.576	---	Transcribed locus	---	---	---
1565915_at	-3.573	---	Full length insert cDNA clone YR04D03	---	---	---
229054_at	-3.511	C14orf181	chromosome 14 open reading frame 181	---	---	---
1558546_at	-3.489	DNASE1	deoxyribonuclease I	---	DNA catabolic process /// apoptosis	actin binding /// nuclease activity /// endonuclease activity /// deoxyribonuclease I activity /// deoxyribonuclease activity /// calcium ion binding /// protein binding /// hydrolase activity
241270_at	-3.47	---	CDNA FLJ39948 fis, clone SPLEN2024273	---	---	---
220234_at	-3.469	CA8	carbonic anhydrase VIII	---	one-carbon compound metabolic process /// phosphoinositide-mediated signaling	carbonate dehydratase activity /// carbonate dehydratase activity /// protein binding /// zinc ion binding /// metal ion binding
207728_at	-3.438	ATF7IP	activating transcription factor 7 interacting protein	---	DNA methylation /// transcription /// regulation of transcription, DNA-dependent /// negative regulation of transcription, DNA-dependent /// positive regulation of transcription, DNA-dependent /// regulation of transcriptional preinitiation complex assembly	protein binding
242430_at	-3.408	CCDC69	Coiled-coil domain containing 69	---	---	---
211696_x_at	-3.399	HBB	hemoglobin, beta	---	transport /// regulation of blood pressure /// oxygen transport /// oxygen transport /// oxygen transport /// nitric oxide transport /// positive regulation of nitric oxide biosynthetic process /// regulation of blood vessel size	oxygen transporter activity /// oxygen transporter activity /// oxygen transporter activity /// iron ion binding /// protein binding /// protein binding /// oxygen binding /// oxygen binding /// heme binding /// hemoglobin binding /// metal ion binding
233708_at	-3.377	---	CDNA: FLJ21147 fis, clone CAS09371	---	---	---
244787_at	-3.369	---	Transcribed locus	---	---	---
243262_at	-3.355	---	---	---	---	---
1561133_at	-3.29	---	Full length insert cDNA clone ZD60A10	---	---	---
229053_at	-3.219	SYT17	Synaptotagmin XVII	---	transport	transporter activity
203206_at	-3.194	FAM53B	family with sequence similarity 53, member B	---	---	---
220214_at	-3.174	ZNF215	zinc finger protein 215	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// transcription factor activity /// zinc ion binding /// zinc ion binding /// metal ion binding
220962_s_at	-3.14	PADI1	peptidyl arginine deiminase, type I	---	protein modification process /// peptidyl-citrulline biosynthetic process from peptidyl-arginine	protein-arginine deiminase activity /// calcium ion binding /// hydrolase activity
1557551_at	-3.137	---	CDNA FLJ13866 fis, clone THYRO1001213	---	---	---
237457_at	-3.121	EIF3B	eukaryotic translation initiation factor 3, subunit B	Translation_Factors	translation /// translational initiation /// translational initiation	nucleotide binding /// nucleic acid binding /// RNA binding /// translation initiation factor activity /// translation initiation factor activity /// protein binding
216171_at	-3.101	---	CDNA: FLJ21618 fis, clone COL07487	---	---	---
1560274_at	-3.09	LOC100132279 /// WTAP	Wilms tumor 1 associated protein /// hypothetical protein LOC100132279	---	mRNA processing /// cell cycle /// RNA splicing	---
204939_s_at	-3.041	PLN	phospholamban	Calcium_regulation_in_cardiac_cells	regulation of the force of heart contraction /// calcium ion transport /// cellular calcium ion homeostasis /// muscle contraction /// blood circulation /// negative regulation of heart contraction /// cardiac muscle development /// regulation of calcium ion transport	calcium channel regulator activity /// protein binding /// protein binding /// ATPase inhibitor activity
1566762_at	-3.019	---	CDNA FLJ23653 fis, clone COL10079	---	---	---
207944_at	-3.004	LOC4951	parvalbumin	---	---	---
242975_s_at	-3	---	Transcribed locus	---	---	---
222161_at	-2.993	NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2	---	proteolysis /// proteolysis	catalytic activity /// carboxypeptidase activity /// carboxypeptidase activity /// dipeptidyl-peptidase IV activity /// peptidase activity /// metallopeptidase activity /// zinc ion binding /// hydrolase activity /// dipeptidase activity /// dipeptidase activity /// glutamate carboxypeptidase II activity /// metal ion binding
231658_x_at	-2.973	---	---	---	---	---
218978_s_at	-2.969	SLC25A37	solute carrier family 25, member 37	---	transport /// ion transport /// iron ion transport /// mitochondrial iron ion transport	iron ion transmembrane transporter activity /// binding /// iron ion binding
219256_s_at	-2.965	SH3TC1	SH3 domain and tetratricopeptide repeats 1	---	---	binding
237379_at	-2.965	KIAA1542	CTD-binding SR-like protein rA9	---	---	protein binding /// zinc ion binding /// metal ion binding
1553220_at	-2.963	ALS2CR13	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 13	---	---	---
243847_at	-2.935	---	Transcribed locus	---	---	---
236164_at	-2.933	---	Transcribed locus	---	---	---
207686_s_at	-2.922	CASP8	caspase 8, apoptosis-related cysteine peptidase	Apoptosis /// Apoptosis_GenMAPP /// Apoptosis_KEGG	proteolysis /// proteolysis /// proteolysis /// apoptosis /// apoptotic program /// apoptotic program /// regulation of apoptosis /// positive regulation of I-kappaB kinase/NF-kappaB cascade	signal transducer activity /// protein binding /// protein binding /// peptidase activity /// cysteine-type peptidase activity /// cysteine-type peptidase activity /// hydrolase activity /// caspase activity /// caspase activity /// identical protein binding
210922_at	-2.908	---	CDNA clone IMAGE:3506689	---	---	---
205500_at	-2.892	C5	complement component 5	Complement_Activation_Classical	activation of MAPK activity /// chemotaxis /// response to stress /// inflammatory response /// inflammatory response /// immune response /// complement activation /// complement activation, alternative pathway /// complement activation, classical pathway /// cell surface receptor linked signal transduction /// G-protein coupled receptor protein signaling pathway /// cytolysis /// innate immune response	endopeptidase inhibitor activity /// receptor binding /// protein binding /// chemokine activity
241150_at	-2.878	SPTAN1	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	---	barbed-end actin filament capping	actin binding /// actin binding /// structural constituent of cytoskeleton /// calcium ion binding /// protein binding /// calmodulin binding

220659_s_at	-2.86	C7orf43	chromosome 7 open reading frame 43	---	---	---
241235_at	-2.843	---	---	---	---	---
1556467_at	-2.831	ZNF80	Zinc finger protein 80	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// zinc ion binding /// metal ion binding
238851_at	-2.823	ANKRD13A	ankyrin repeat domain 13A	---	---	---
221604_s_at	-2.785	PEX16	peroxisomal biogenesis factor 16	---	protein targeting to peroxisome /// peroxisome organization and biogenesis /// peroxisome organization and biogenesis /// peroxisome membrane biogenesis /// protein import into peroxisome matrix /// ER-dependent peroxisome biogenesis /// protein import into peroxisome membrane	protein C-terminus binding
215299_x_at	-2.776	SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	---	catecholamine metabolic process /// lipid metabolic process /// steroid metabolic process /// amine metabolic process	aryl sulfotransferase activity /// sulfotransferase activity /// sulfotransferase activity /// transferase activity
232929_at	-2.774	---	CDNA FLJ13240 fis, clone OVARC1000496	---	---	---
243478_at	-2.774	---	CDNA FLJ40061 fis, clone TESOP2000083	---	---	---
236609_at	-2.768	LOC100129592	Hypothetical protein LOC100129592	---	---	---
213045_at	-2.766	MAST3	microtubule associated serine/threonine kinase 3	---	protein amino acid phosphorylation	nucleotide binding /// magnesium ion binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein binding /// protein binding /// ATP binding /// kinase activity /// transferase activity
227009_at	-2.75	---	CDNA: FLJ21600 fis, clone COL07202	---	---	---
236131_at	-2.75	RHOJ	ras homolog gene family, member J	---	small GTPase mediated signal transduction /// Rho protein signal transduction /// regulation of cell shape /// actin cytoskeleton organization and biogenesis /// actin cytoskeleton organization and biogenesis	nucleotide binding /// GTPase activity /// protein binding /// protein binding /// GTP binding
1567067_s_at	-2.721	LUC7L	LUC7-like (S. cerevisiae)	---	---	protein binding /// zinc ion binding /// metal ion binding
204964_s_at	-2.721	SSPN	sarcospan (Kras oncogene-associated gene)	---	muscle contraction /// cell adhesion	---
238348_x_at	-2.717	---	Transcribed locus	---	---	---
226877_at	-2.702	TYSND1	trypsin domain containing 1	---	proteolysis	catalytic activity /// serine-type endopeptidase activity /// peptidase activity /// hydrolase activity
239404_at	-2.701	---	Transcribed locus	---	---	---
1570033_at	-2.692	WIPI2	WD repeat domain, phosphoinositide interacting 2	---	---	---
205025_at	-2.686	ZBTB48	zinc finger and BTB domain containing 48	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// protein binding /// zinc ion binding /// metal ion binding
215737_x_at	-2.676	USF2	upstream transcription factor 2, c-fos interacting	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// RNA polymerase II transcription factor activity /// protein binding /// transcription regulator activity /// identical protein binding
236974_at	-2.668	---	CDNA clone IMAGE:5265193	---	---	---
1557438_at	-2.665	---	CDNA clone IMAGE:5285425	---	---	---
235757_at	-2.644	---	Transcribed locus	---	---	---
217687_at	-2.643	ADCY2	adenylate cyclase 2 (brain)	Calcium_regulation_in_cardiac_cells /// G_Protein_Signaling /// Smooth_muscle_contraction	cAMP biosynthetic process /// cAMP biosynthetic process /// intracellular signaling cascade /// cyclic nucleotide biosynthetic process	magnesium ion binding /// adenylate cyclase activity /// adenylate cyclase activity /// lyase activity /// phosphorus-oxygen lyase activity /// metal ion binding
242438_at	-2.626	ASXL1	additional sex combs like 1 (Drosophila)	---	transcription /// regulation of transcription, DNA-dependent	zinc ion binding /// metal ion binding
242143_at	-2.62	---	Transcribed locus	---	---	---
211435_at	-2.618	---	---	---	---	---
241491_at	-2.598	---	Transcribed locus	---	---	---
1557066_at	-2.587	LUC7L	LUC7-like (S. cerevisiae)	---	---	protein binding /// zinc ion binding /// metal ion binding
236459_at	-2.583	PRKCE	Protein kinase C, epsilon	Calcium_regulation_in_cardiac_cells /// G_Protein_Signaling /// Smooth_muscle_contraction /// Wnt_signaling	protein amino acid phosphorylation /// protein amino acid phosphorylation /// induction of apoptosis /// signal transduction /// intracellular signaling cascade	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// protein kinase C activity /// protein kinase C activity /// signal transducer activity /// ATP binding /// zinc ion binding /// kinase activity /// transferase activity /// diacylglycerol binding /// metal ion binding
208372_s_at	-2.548	LIMK1	LIM domain kinase 1	G13_Signaling_Pathway	protein amino acid phosphorylation /// protein amino acid phosphorylation /// cell motility /// signal transduction /// Rho protein signal transduction /// nervous system development /// actin cytoskeleton organization and biogenesis /// positive regulation of axon extension	nucleotide binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein tyrosine kinase activity /// protein binding /// protein binding /// ATP binding /// zinc ion binding /// kinase activity /// transferase activity /// metal ion binding /// protein heterodimerization activity
215652_s_at	-2.546	ESR1	estrogen receptor 1	Nuclear_Receptors	transcription /// transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// signal transduction /// estrogen receptor signaling pathway /// neuroprotection /// positive regulation of survival gene product expression	DNA binding /// transcription factor activity /// steroid hormone receptor activity /// steroid hormone receptor activity /// steroid hormone receptor activity /// receptor activity /// ligand-dependent nuclear receptor activity /// steroid binding /// protein binding /// zinc ion binding /// lipid binding /// nitric-oxide synthase regulator activity /// estrogen receptor activity /// hormone binding /// sequence-specific DNA binding /// metal ion binding /// protein N-terminus binding
236357_at	-2.544	LOC100129951	hypothetical protein LOC100129951	---	---	---
200719_at	-2.543	SKP1	S-phase kinase-associated protein 1	---	ubiquitin-dependent protein catabolic process /// ubiquitin cycle	protein binding /// protein binding
1568534_at	-2.492	DKFZp547E087	hypothetical gene LOC283846	---	---	---
220954_s_at	-2.491	PILRB	paired immunoglobin-like type 2 receptor beta	---	transmembrane receptor protein tyrosine kinase signaling pathway /// activation of transmembrane receptor protein tyrosine kinase activity	receptor activity /// protein binding
240806_at	-2.466	RPL15	Ribosomal protein L15	Ribosomal_Proteins /// Ribosomal_Proteins	translation /// translation	RNA binding /// structural constituent of ribosome /// structural constituent of ribosome
241762_at	-2.462	FBXO32	F-box protein 32	---	ubiquitin cycle	protein binding
211581_x_at	-2.456	LST1	leukocyte specific transcript 1	---	cell morphogenesis /// immune response /// immune response /// regulation of cell shape /// anatomical structure morphogenesis /// dendrite development /// negative regulation of lymphocyte proliferation	protein binding
204249_s_at	-2.448	LMO2	LIM domain only 2 (rhombotin-like 1)	---	multicellular organismal development	protein binding /// zinc ion binding /// metal ion binding
1561600_at	-2.44	---	MRNA; cDNA DKFZp586O2224 (from clone DKFZp586O2224)	---	---	---
240789_at	-2.438	---	---	---	---	---
213228_at	-2.429	PDE8B	phosphodiesterase 8B	G_Protein_Signaling	two-component signal transduction system (phosphorelay) /// regulation of transcription, DNA-dependent /// signal transduction /// cyclic nucleotide metabolic process	two-component response regulator activity /// magnesium ion binding /// catalytic activity /// 3',5'-cyclic-nucleotide phosphodiesterase activity /// 3',5'-cyclic-nucleotide phosphodiesterase activity /// signal transducer activity /// hydrolase activity /// manganese ion binding /// metal ion binding
202315_s_at	-2.423	BCR	breakpoint cluster region	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// signal transduction /// signal transduction /// intracellular signaling cascade /// intracellular signaling cascade /// regulation of Rho protein signal transduction	protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// protein serine/threonine kinase activity /// guanyl-nucleotide exchange factor activity /// Rho guanyl-nucleotide exchange factor activity /// GTPase activator activity /// GTPase activator activity /// GTPase activator activity /// kinase activity /// transferase activity
221288_at	-2.418	GPR22	G protein-coupled receptor 22	---	signal transduction /// G-protein coupled receptor protein signaling pathway /// G-protein coupled receptor protein signaling pathway	rhodopsin-like receptor activity /// signal transducer activity /// receptor activity /// G-protein coupled receptor activity /// G-protein coupled receptor activity
1556417_a_at	-2.402	---	Full length insert cDNA clone YP01H07	---	---	---
213378_s_at	-2.398	DDX11 /// DDX12 /// LOC642846	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae) /// DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 12 (CHL1-like helicase homolog, S. cerevisiae) /// DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11-like	---	mitotic sister chromatid segregation /// S phase of mitotic cell cycle /// G2/M transition of mitotic cell cycle /// nucleobase, nucleoside, nucleotide and nucleic acid metabolic process /// cell cycle /// positive regulation of cell proliferation	nucleotide binding /// nucleic acid binding /// DNA binding /// DNA helicase activity /// RNA binding /// ATP-dependent DNA helicase activity /// helicase activity /// helicase activity /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity /// hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides

231768_at	-2.338	USF1	upstream transcription factor 1	---	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// regulation of transcription	DNA binding /// specific RNA polymerase II transcription factor activity /// protein binding /// transcription regulator activity
204141_at	-2.337	TUBB2A	tubulin, beta 2A	---	microtubule-based process /// microtubule-based movement /// mitosis /// neuron differentiation /// protein polymerization	nucleotide binding /// GTPase activity /// structural molecule activity /// structural constituent of cytoskeleton /// protein binding /// GTP binding
234393_at	-2.323	HDAC9	histone deacetylase 9	---	transcription /// regulation of transcription, DNA-dependent /// inflammatory response /// chromatin modification /// histone deacetylation /// B cell differentiation /// B cell activation /// negative regulation of striated muscle development	histone deacetylase activity /// protein binding /// protein binding /// transcription factor binding /// specific transcriptional repressor activity /// hydrolase activity
232483_at	-2.32	MED17	mediator complex subunit 17	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// regulation of transcription from RNA polymerase II promoter /// transcription initiation from RNA polymerase II promoter /// transcription initiation from RNA polymerase II promoter /// steroid hormone receptor signaling pathway /// androgen receptor signaling pathway /// positive regulation of transcription from RNA polymerase II promoter	transcription cofactor activity /// transcription coactivator activity /// transcription coactivator activity /// receptor activity /// protein binding /// RNA polymerase II transcription mediator activity /// transcription activator activity /// ligand-dependent nuclear receptor transcription coactivator activity /// transcription regulator activity /// vitamin D receptor binding /// thyroid hormone receptor binding
1556620_at	-2.316	---	Full length insert cDNA clone YT69G03	---	---	---
236405_at	-2.315	GSTA4	glutathione S-transferase A4	---	response to stress /// metabolic process	glutathione transferase activity /// glutathione transferase activity /// glutathione transferase activity /// transferase activity
216211_at	-2.308	---	MRNA; cDNA DKFZp564A023 (from clone DKFZp564A023)	---	---	---
209836_x_at	-2.302	BOLA2 /// BOLA2B	bolA homolog 2 (E. coli) /// bolA homolog 2B (E. coli)	---	---	---
239627_at	-2.279	RAB3GAP1	RAB3 GTPase activating protein subunit 1 (catalytic)	---	regulation of GTPase activity	GTPase activator activity /// Rab GTPase activator activity /// Rab GTPase binding
241907_at	-2.253	---	Full length insert cDNA clone YR58A06	---	---	---
211996_s_at	-2.247	DKFZp547E087 /// LOC100132247 /// LOC23117 /// LOC348162 /// LOC440345 /// LOC613037	KIAA0220-like protein /// hypothetical gene LOC283846 /// hypothetical protein 348162 /// hypothetical protein LOC440345 /// nuclear pore complex interacting protein pseudogene /// similar to Uncharacterized protein KIAA0220	---	---	binding
229415_at	-2.234	CYCS	cytochrome c, somatic	Apoptosis /// Apoptosis_GenMAPP /// Apoptosis_KEGG /// Electron_Transport_Chain	DNA fragmentation during apoptosis /// transport /// apoptosis /// apoptosis /// caspase activation via cytochrome c /// cellular respiration /// oxidation reduction	protein serine/threonine phosphatase activity /// iron ion binding /// protein binding /// electron carrier activity /// heme binding /// heme binding /// electron transporter, transferring electrons from CoQH2-cytochrome c reductase complex and cytochrome c oxidase complex activity /// metal ion binding
242431_at	-2.221	---	---	---	---	---
204990_s_at	-2.206	ITGB4	integrin, beta 4	Integrin-mediated_cell_adhesion_KEGG	cell communication /// cell adhesion /// cell adhesion /// cell-matrix adhesion /// integrin-mediated signaling pathway /// multicellular organismal development	receptor activity /// binding /// protein binding /// protein binding
234735_s_at	-2.205	USP21	ubiquitin specific peptidase 21	---	protein modification process /// ubiquitin-dependent protein catabolic process /// ubiquitin cycle	ubiquitin thiolesterase activity /// ubiquitin thiolesterase activity /// protein binding /// peptidase activity /// cysteine-type peptidase activity /// hydrolase activity
241988_x_at	-2.185	---	Transcribed locus, weakly similar to XP_001091208.1 PREDICTED: hypothetical protein [Macaca mulatta]	---	---	---
232498_at	-2.172	KIAA1833	hypothetical protein KIAA1833	---	---	binding
81737_at	-2.171	---	Homo sapiens, clone IMAGE:4271781	---	---	---
224385_s_at	-2.17	MOV10L1	Mov10l1, Moloney leukemia virus 10-like 1, homolog (mouse)	---	multicellular organismal development /// germ cell development /// spermatogenesis	nucleotide binding /// magnesium ion binding /// RNA binding /// ATP-dependent RNA helicase activity /// helicase activity /// ATP binding /// ATP binding /// hydrolase activity
204793_at	-2.162	GPRASP1	G protein-coupled receptor associated sorting protein 1	---	---	binding
206742_at	-2.161	FIGF	c-fos induced growth factor (vascular endothelial growth factor D)	---	angiogenesis /// multicellular organismal development /// cell proliferation /// positive regulation of cell proliferation /// positive regulation of cell proliferation /// cell differentiation /// vascular endothelial growth factor receptor signaling pathway	receptor binding /// platelet-derived growth factor receptor binding /// protein binding /// growth factor activity /// protein homodimerization activity /// vascular endothelial growth factor receptor 3 binding
227543_at	-2.159	RNASEH2C	ribonuclease H2, subunit C	---	---	---
232321_at	-2.159	MUC17	mucin 17, cell surface associated	---	---	nucleic acid binding /// zinc ion binding /// extracellular matrix constituent, lubricant activity
33307_at	-2.157	CTA-126B4.3	CGI-96 protein	---	---	nucleic acid binding /// RNA binding
210150_s_at	-2.127	LAMA5	laminin, alpha 5	Inflammatory_Response_Pathway	angiogenesis /// cytoskeleton organization and biogenesis /// cell adhesion /// integrin-mediated signaling pathway /// cell recognition /// cell proliferation /// embryonic development /// cell migration /// cell differentiation /// regulation of cell adhesion /// regulation of cell migration /// endothelial cell differentiation /// regulation of embryonic development /// focal adhesion formation	receptor activity /// receptor binding /// integrin binding /// structural molecule activity /// structural molecule activity /// protein binding
216407_at	-2.122	VAC14	Vac14 homolog (S. cerevisiae)	---	signal transduction	receptor activity /// binding
203802_x_at	-2.099	NSUN5	NOL1/NOP2/Sun domain family, member 5	---	---	methyltransferase activity /// transferase activity
239813_at	-2.098	IQCH	IQ motif containing H	---	---	---
201248_s_at	-2.096	SREBF2	sterol regulatory element binding transcription factor 2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// lipid metabolic process /// lipid metabolic process /// steroid metabolic process /// cholesterol metabolic process /// regulation of transcription	DNA binding /// transcription factor activity /// RNA polymerase II transcription factor activity /// protein binding /// protein binding /// transcription regulator activity
1556060_a_at	-2.095	KIAA1702	KIAA1702 protein	---	phosphoenolpyruvate-dependent sugar phosphotransferase system	sugar:hydrogen symporter activity
1557737_s_at	-2.086	NKTR	natural killer-tumor recognition sequence	---	protein folding	peptidyl-prolyl cis-trans isomerase activity /// cyclosporin A binding /// isomerase activity /// peptide binding
220401_at	-2.07	FLJ21369	hypothetical protein FLJ21369	---	---	---
1557232_at	-2.069	---	CDNA clone IMAGE:4797260	---	---	---
232612_s_at	-2.064	ATG16L1	ATG16 autophagy related 16-like 1 (S. cerevisiae)	---	transport /// autophagy /// protein transport	protein binding
222145_at	-2.061	---	CDNA: FLJ23572 fis, clone LNG12403	---	---	---
231545_at	-2.061	---	---	---	---	---
220205_at	-2.06	TPTE	transmembrane phosphatase with tensin homology	---	protein amino acid dephosphorylation /// signal transduction	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// protein tyrosine phosphatase activity /// hydrolase activity
204978_at	-2.051	SFRS16	splicing factor, arginine/serine-rich 16	mRNA_processing_Reactome	mRNA processing /// RNA splicing	---
236023_at	-2.04	CDK9	cyclin-dependent kinase 9	---	transcription /// regulation of transcription, DNA-dependent /// transcription initiation from RNA polymerase II promoter /// RNA elongation from RNA polymerase II promoter /// protein amino acid phosphorylation /// protein amino acid phosphorylation /// cell proliferation	nucleotide binding /// DNA binding /// protein kinase activity /// protein kinase activity /// protein serine/threonine kinase activity /// cyclin-dependent protein kinase activity /// cyclin-dependent protein kinase activity /// protein binding /// ATP binding /// RNA polymerase subunit kinase activity /// kinase activity /// transferase activity /// snRNA binding
224283_x_at	-2.03	IL18BP	interleukin 18 binding protein	---	T-helper 1 type immune response	interleukin-18 binding /// receptor antagonist activity
228147_at	-2.025	---	---	---	---	---
233809_at	-2.008	HYPK	Huntingtin interacting protein K	---	---	protein binding
234589_at	-2.006	TMEM106A	Transmembrane protein 106A	---	---	---
206121_at	-1.994	AMPD1	adenosine monophosphate deaminase 1 (isoform M)	---	nucleotide metabolic process /// purine ribonucleoside monophosphate biosynthetic process	AMP deaminase activity /// AMP deaminase activity /// hydrolase activity /// deaminase activity
240609_at	-1.979	---	Transcribed locus	---	---	---
203653_s_at	-1.977	COIL	coilin	---	---	protein binding /// protein binding /// protein C-terminus binding /// protein C-terminus binding /// disulfide oxidoreductase activity
227524_at	-1.973	---	CDNA clone IMAGE:5311370	---	---	---
240282_at	-1.972	WDR1	WD repeat domain 1	Hypertrophy_model	sensory perception of sound	actin binding /// protein binding /// protein binding
222861_x_at	-1.961	FBXO44	F-box protein 44	---	ubiquitin cycle /// protein catabolic process	protein binding
231838_at	-1.961	PABPC1L	poly(A) binding protein, cytoplasmic 1-like	---	mRNA metabolic process	nucleotide binding /// nucleic acid binding /// RNA binding
242932_at	-1.946	---	Transcribed locus	---	---	---

240341_at	-1.945	---	Transcribed locus	---	---	---
237972_at	-1.928	---	---	---	---	---
239882_at	-1.919	---	Non-coding transcript, polyA signal, clone 48-E/2.4Kb	---	---	---
238068_at	-1.916	ARIH2	Ariadne homolog 2 (Drosophila)	---	ubiquitin cycle /// multicellular organismal development	nucleic acid binding /// protein binding /// zinc ion binding /// zinc ion binding /// metal ion binding
236832_at	-1.912	LOC221442	hypothetical LOC221442	---	---	---
228475_at	-1.903	CCDC61	Coiled-coil domain containing 61	---	---	---
232811_x_at	-1.892	PRICKLE1	Prickle homolog 1 (Drosophila)	---	---	zinc ion binding /// metal ion binding
1568596_a_at	-1.888	TROAP	trophinin associated protein (tastin)	---	cell adhesion /// cell adhesion	protein binding
211751_at	-1.865	PDE4DIP	phosphodiesterase 4D interacting protein (myomegalin)	---	cytoskeleton organization and biogenesis /// actin cytoskeleton organization and biogenesis	actin binding
205544_s_at	-1.864	CR2	complement component (3d/Epstein Barr virus) receptor 2	---	immune response /// immune response /// complement activation, classical pathway /// innate immune response	receptor activity /// complement receptor activity /// complement receptor activity /// transmembrane receptor activity /// protein homodimerization activity
237488_at	-1.855	---	---	---	---	---
231988_x_at	-1.846	ZNF490	zinc finger protein 490	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
1568815_a_at	-1.821	DDX50	DEAD (Asp-Glu-Ala-Asp) box polypeptide 50	---	---	nucleotide binding /// nucleic acid binding /// RNA binding /// helicase activity /// ATP binding /// ATP-dependent helicase activity /// hydrolase activity
233089_at	-1.814	QRSL1	glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1	---	translation	ligase activity /// carbon-nitrogen ligase activity, with glutamine as amido-N-donor
234675_x_at	-1.813	---	CDNA: FLJ23566 fis, clone LNG10880	---	---	---
219807_x_at	-1.801	RAB4B	RAB4B, member RAS oncogene family	---	transport /// small GTPase mediated signal transduction /// protein transport /// vesicle-mediated transport	nucleotide binding /// GTPase activity /// GTP binding
228460_at	-1.801	ZNF319	zinc finger protein 319	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// zinc ion binding /// metal ion binding
242642_at	-1.786	---	---	---	---	---
243186_at	-1.766	---	---	---	---	---
215134_at	-1.754	PI4K2A	phosphatidylinositol 4-kinase type 2 alpha	---	phosphatidylinositol biosynthetic process	magnesium ion binding /// 1-phosphatidylinositol 4-kinase activity /// 1-phosphatidylinositol 4-kinase activity /// protein binding /// kinase activity /// transferase activity /// phosphotransferase activity, alcohol group as acceptor
208686_s_at	-1.734	BRD2	bromodomain containing 2	---	spermatogenesis	protein serine/threonine kinase activity
222998_at	-1.727	MAF1	MAF1 homolog (S. cerevisiae)	---	transcription /// regulation of transcription, DNA-dependent /// negative regulation of transcription from RNA polymerase III promoter	transcription regulator activity
242855_at	-1.7	CRIM2	cysteine rich BMP regulator 2 (chordin-like)	---	---	---
239959_x_at	-1.697	---	---	---	---	---
1554957_at	-1.682	---	---	---	---	---
233042_at	-1.669	C1S	Complement component 1, s subcomponent	Complement_Activation_Classical	proteolysis /// immune response /// complement activation, classical pathway /// G-protein coupled receptor protein signaling pathway /// innate immune response	rhodopsin-like receptor activity /// complement component C1s activity /// complement component C1s activity /// catalytic activity /// serine-type endopeptidase activity /// calcium ion binding /// peptidase activity /// serine-type peptidase activity /// hydrolase activity /// metal ion binding
230632_at	-1.666	---	CDNA FLJ41728 fis, clone HLUNG2015617	---	---	---
210720_s_at	-1.656	NECAB3	N-terminal EF-hand calcium binding protein 3	---	protein secretion /// antibiotic biosynthetic process /// protein metabolic process /// protein metabolic process /// regulation of amyloid precursor protein biosynthetic process	calcium ion binding /// protein binding /// protein binding /// protein binding /// oxidoreductase activity
230270_at	-1.64	PRPF38B	PRP38 pre-mRNA processing factor 38 (yeast) domain containing B	---	mRNA processing /// RNA splicing	---
217614_at	-1.635	---	Transcribed locus	---	---	---
1555436_a_at	-1.63	AFF4	AF4/FMR2 family, member 4	---	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter	transcription factor activity
1557248_at	-1.629	ZNF687	Zinc finger protein 687	---	transcription /// regulation of transcription, DNA-dependent /// post-translational protein modification /// regulation of protein metabolic process	nucleic acid binding /// DNA binding /// zinc ion binding /// small conjugating protein ligase activity /// metal ion binding
237504_at	-1.627	INTS10	integrator complex subunit 10	---	snRNA processing	protein binding
223243_s_at	-1.622	EDEM3	ER degradation enhancer, mannosidase alpha-like 3	---	glycoprotein catabolic process /// response to unfolded protein /// proteasomal ubiquitin-dependent protein catabolic process	glycoprotein endo-alpha-1,2-mannosidase activity /// mannosyl-oligosaccharide 1,2-alpha-mannosidase activity /// calcium ion binding
207330_at	-1.611	PZP	pregnancy-zone protein	---	female pregnancy	endopeptidase inhibitor activity /// endopeptidase inhibitor activity /// serine-type endopeptidase inhibitor activity /// protein binding /// wide-spectrum protease inhibitor activity
206646_at	-1.603	GLI1	glioma-associated oncogene homolog 1 (zinc finger protein)	---	osteoblast differentiation /// transcription /// regulation of transcription, DNA-dependent /// multicellular organismal development /// spermatogenesis /// ventral midline development /// positive regulation of cell proliferation /// regulation of smoothened signaling pathway /// epidermal cell differentiation /// dorsal/ventral pattern formation /// proximal/distal pattern formation /// cerebellar cortex morphogenesis /// smoothened signaling pathway in regulation of granule cell precursor cell proliferation /// pituitary gland development /// cell differentiation /// lung development /// positive regulation of DNA replication /// positive regulation of transcription from RNA polymerase II promoter /// positive regulation of transcription from RNA polymerase II promoter /// notochord regression	nucleic acid binding /// DNA binding /// DNA binding /// chromatin binding /// protein binding /// protein binding /// microtubule binding /// zinc ion binding /// transcription activator activity /// transcription activator activity /// metal ion binding
1555255_a_at	-1.598	HISPPD2A	histidine acid phosphatase domain containing 2A	---	inositol metabolic process	inositol 1,3,4,5,6-pentakisphosphate kinase activity /// inositol hexakisphosphate 5-kinase activity /// acid phosphatase activity /// kinase activity /// transferase activity /// diphosphoinositol-pentakisphosphate kinase activity /// diphosphoinositol-pentakisphosphate kinase activity
233004_x_at	-1.596	---	CDNA FLJ11855 fis, clone HEMBA1006780	---	---	---
236015_at	-1.594	---	---	---	---	---
228180_at	-1.579	---	Transcribed locus	---	---	---
242125_at	-1.568	---	CDNA FLJ36209 fis, clone THYMU2000022	---	---	---
231793_s_at	-1.558	CAMK2D	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	Calcium_regulation_in_cardiac_cells /// Smooth_muscle_contraction	regulation of cell growth /// protein amino acid phosphorylation /// protein amino acid phosphorylation	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// calmodulin-dependent protein kinase activity /// protein binding /// calmodulin binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity
229099_at	-1.553	LOC790955	hypothetical protein LOC790955	---	---	---
233101_at	-1.553	MTMR9	myotubularin related protein 9	---	phospholipid dephosphorylation	inositol or phosphatidylinositol phosphatase activity /// protein binding /// enzyme regulator activity
212520_s_at	-1.548	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	---	negative regulation of transcription from RNA polymerase II promoter /// blastocyst growth /// blastocyst hatching /// methylation-dependent chromatin silencing /// transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// glial cell fate determination /// forebrain development /// hindbrain development	nucleotide binding /// nucleic acid binding /// DNA binding /// chromatin binding /// transcription factor activity /// transcription coactivator activity /// helicase activity /// helicase activity /// protein binding /// ATP binding /// transcription factor binding /// hydrolase activity /// identical protein binding /// protein N-terminus binding
238446_at	-1.545	SMA4	Glucuronidase, beta pseudogene 1	---	skeletal development /// carbohydrate metabolic process /// nervous system development	catalytic activity /// hydrolase activity, hydrolyzing O-glycosyl compounds /// cation binding
231904_at	-1.538	U2AF1	U2 small nuclear RNA auxiliary factor 1	mRNA_processing_Reactome	nuclear mRNA splicing, via spliceosome /// mRNA processing /// mRNA processing /// RNA splicing /// RNA splicing	nucleotide binding /// nucleic acid binding /// RNA binding /// protein binding /// zinc ion binding /// metal ion binding
212381_at	-1.529	USP24	ubiquitin specific peptidase 24	---	ubiquitin-dependent protein catabolic process /// ubiquitin cycle	ubiquitin thiolesterase activity /// peptidase activity /// cysteine-type peptidase activity /// hydrolase activity
228239_at	-1.509	C21orf51	chromosome 21 open reading frame 51	---	---	---
1556778_at	-1.506	---	Full length insert cDNA clone YZ87H02	---	---	---
239400_at	-1.49	FLJ45513	hypothetical LOC729220	---	---	---
211136_s_at	-1.474	CLPTM1	cleft lip and palate associated transmembrane protein 1	---	multicellular organismal development /// multicellular organismal development /// cell differentiation /// regulation of T cell differentiation in the thymus	---
235577_at	-1.47	ZNF652	Zinc finger protein 652	---	transcription /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// protein binding /// zinc ion binding /// metal ion binding
235239_at	-1.443	QSOX2	quiescin Q6 sulfhydryl oxidase 2	---	---	oxidoreductase activity /// thiol oxidase activity

9.3. Genes regulated in both, naïve and memory B cells upon EBV transformation

GENES DOWNREGULATED EXCLUSIVELY IN BOTH, NAIVE AND MEMORY B CELLS UPON TRANSFORMATION WITH EBV

Probe Set ID	Fold Change	Gene Symbol	Gene Title	Pathway	go biological process term	go molecular function term
229656_s_at	-79.19	FLJ42562	similar to echinoderm microtubule associated protein like 5	---	---	---
241446_at	-55.58	ADAM28	ADAM metallopeptidase domain 28	---	proteolysis /// spermatogenesis	metalloendopeptidase activity /// peptidase activity /// metallopeptidase activity /// metallopeptidase activity /// zinc ion binding /// hydrolase activity /// metal ion binding
221234_s_at	-40.61	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// protein binding /// sequence-specific DNA binding /// protein dimerization activity
231093_at	-26.88	FCRL3	Fc receptor-like 3	---	methylation	nucleic acid binding /// receptor activity /// methyltransferase activity
1559618_at	-26.78	LOC100129447	hypothetical protein LOC100129447	---	---	---
219073_s_at	-26.59	OSBPL10	oxysterol binding protein-like 10	---	transport /// lipid transport /// steroid metabolic process	---
234217_at	-25.5	---	CDNA: FLJ21283 fis, clone COL01910	---	---	---
242388_x_at	-19.37	---	Transcribed locus	---	---	---
1563674_at	-19.13	FCRL2	Fc receptor-like 2	---	cell-cell signaling	receptor activity /// SH3/SH2 adaptor activity
205671_s_at	-18.86	HLA-DOB	major histocompatibility complex, class II, DO beta	---	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II /// immune response /// antigen processing and presentation	nucleotide binding /// ATP binding /// MHC class II receptor activity /// peptide binding /// MHC class I protein binding
239901_at	-18.86	---	Transcribed locus	---	---	---
235490_at	-18.44	TMEM107	transmembrane protein 107	---	---	---
235683_at	-8.835	SESN3	sestrin 3	---	cell cycle arrest	---
224367_at	-8.008	BEX2	brain expressed X-linked 2	---	---	---
209771_x_at	-7.898	CD24	CD24 molecule	---	response to hypoxia /// cell activation /// regulation of cytokine and chemokine mediated signaling pathway /// regulation of cytokine and chemokine mediated signaling pathway /// response to molecule of bacterial origin /// response to molecule of bacterial origin /// immune response-regulating cell surface receptor signaling pathway /// elevation of cytosolic calcium ion concentration /// neuromuscular synaptic transmission /// induction of apoptosis by intracellular signals /// Wnt receptor signaling pathway /// cell-cell adhesion /// cell migration /// cell migration /// regulation of epithelial cell differentiation /// T cell costimulation /// B cell receptor transport into membrane raft /// chemokine receptor transport out of membrane raft /// negative regulation of transforming growth factor-beta3 production /// positive regulation of activated T cell proliferation /// regulation of phosphorylation /// cholesterol homeostasis /// cholesterol homeostasis /// positive regulation of MAP kinase activity /// regulation of MAPKKK cascade /// response to estrogen stimulus /// respiratory burst /...	signal transducer activity /// protein binding /// protein binding /// protein kinase binding /// carbohydrate binding /// protein tyrosine kinase activator activity
204440_at	-7.767	CD83	CD83 molecule	---	defense response /// humoral immune response /// signal transduction	---
242906_at	-7.491	---	Transcribed locus	---	---	---
230128_at	-7.371	IGL@	Immunoglobulin lambda joining 3	---	tRNA aminoacylation for protein translation	nucleotide binding /// aminoacyl-tRNA ligase activity /// ATP binding
1554508_at	-6.161	PIK3AP1	phosphoinositide-3-kinase adaptor protein 1	---	---	kinase activity
1566079_at	-5.08	LOC647190	similar to 40S ribosomal protein S16	---	translation	structural constituent of ribosome
218935_at	-5.064	EHD3	EH-domain containing 3	---	---	nucleotide binding /// nucleic acid binding /// GTPase activity /// calcium ion binding /// ATP binding /// GTP binding
228056_s_at	-4.547	NAPSB	napsin B aspartic peptidase pseudogene	---	proteolysis /// proteolysis	aspartic-type endopeptidase activity /// pepsin A activity /// pepsin A activity /// peptidase activity /// hydrolase activity
1552807_a_at	-4.519	SIGLEC10	sialic acid binding Ig-like lectin 10	---	cell adhesion	protein binding /// sugar binding
240144_at	-4.351	DNASE1	deoxyribonuclease I	---	DNA catabolic process /// apoptosis	actin binding /// endonuclease activity /// deoxyribonuclease I activity /// deoxyribonuclease activity /// calcium ion binding /// protein binding /// hydrolase activity
217242_at	-3.49	ZNF154	zinc finger protein 154	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent	nucleic acid binding /// DNA binding /// transcription factor activity /// zinc ion binding /// metal ion binding
1556202_at	-3.403	SRGAP2	SLIT-ROBO Rho GTPase activating protein 2	---	signal transduction	GTPase activator activity
202786_at	-3.271	STK39	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	---	protein amino acid phosphorylation /// protein amino acid phosphorylation /// response to stress	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// receptor signaling protein serine/threonine kinase activity /// protein tyrosine kinase activity /// protein binding /// ATP binding /// ATP binding /// kinase activity /// transferase activity
242292_at	-3.212	CXorf50 /// hCG_1731871 /// LOC100132401	chromosome X open reading frame 50 /// hCG1731871 /// hypothetical protein LOC100132401	---	---	---
238071_at	-3.131	LCN10	lipocalin 10	---	transport	transporter activity /// binding
244519_at	-2.449	ASXL1	additional sex combs like 1 (Drosophila)	---	transcription /// regulation of transcription, DNA-dependent	zinc ion binding /// metal ion binding
203385_at	-2.174	DGKA	diacylglycerol kinase, alpha 80kDa	---	activation of protein kinase C activity /// intracellular signaling cascade /// intracellular signaling cascade	diacylglycerol kinase activity /// diacylglycerol kinase activity /// diacylglycerol kinase activity /// calcium ion binding /// phospholipid binding /// zinc ion binding /// kinase activity /// transferase activity /// diacylglycerol binding /// metal ion binding
215364_s_at	-2.108	KIAA0467	KIAA0467	---	---	---
207100_s_at	-1.89	VAMP1	vesicle-associated membrane protein 1 (synaptobrevin 1)	---	vesicle-mediated transport	protein binding
214879_x_at	-1.822	USF2	upstream transcription factor 2, c-fos interacting	---	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription	DNA binding /// transcription factor activity /// RNA polymerase II transcription factor activity /// protein binding /// transcription regulator activity /// identical protein binding

GENES UPREGULATED EXCLUSIVELY IN BOTH, NAIVE AND MEMORY B CELLS UPON TRANSFORMATION WITH EBV

Probe Set ID	Fold Change	Gene Symbol	Gene Title	Pathway	go biological process term	go molecular function term
1556499_s_at	337.8	COL1A1	collagen, type I, alpha 1	Inflammatory_Response_Pathway	skeletal development /// ossification /// phosphate transport /// response to nutrient /// sensory perception of sound /// epidermis development /// response to mechanical stimulus /// response to inorganic substance /// response to corticosteroid stimulus /// response to hydrogen peroxide /// response to peptide hormone stimulus /// response to cAMP	structural molecule activity /// extracellular matrix structural constituent /// protein binding /// structural constituent of bone
202403_s_at	81.09	COL1A2	collagen, type I, alpha 2	---	skeletal development /// phosphate transport /// transmembrane receptor protein tyrosine kinase signaling pathway /// sensory perception of sound	structural molecule activity /// extracellular matrix structural constituent /// extracellular matrix structural constituent /// protein binding /// structural constituent of bone
212097_at	69.93	CAV1	caveolin 1, caveolae protein, 22kDa	Integrin-mediated_cell_adhesion_KEGG	inactivation of MAPK activity /// vasculogenesis /// response to hypoxia /// negative regulation of endothelial cell proliferation /// triacylglycerol metabolic process /// calcium ion transport /// cellular calcium ion homeostasis /// endocytosis /// regulation of smooth muscle contraction /// skeletal muscle development /// protein localization /// vesicle organization and biogenesis /// regulation of fatty acid metabolic process /// sequestering of lipid /// regulation of blood coagulation /// cholesterol transport /// negative regulation of epithelial cell differentiation /// mammary gland development /// nitric oxide homeostasis /// cholesterol homeostasis /// cholesterol homeostasis /// negative regulation of MAPKKK cascade /// negative regulation of nitric oxide biosynthetic process /// positive regulation of vasoconstriction /// negative regulation of vasodilation /// negative regulation of JAK-STAT cascade /// positive regulation of metalloenzyme activity /// protein homooligomerization /// membrane depolarization /// regulation of peptidase activity /// calcium ion homeostasis /// mammary gland involution	structural molecule activity /// protein binding /// cholesterol binding /// protease activator activity /// nitric-oxide synthase binding
201976_s_at	45.78	MYO10	myosin X	---	signal transduction	nucleotide binding /// motor activity /// actin binding /// binding /// ATP binding
216693_x_at	26.37	HDGFRP3	hepatoma-derived growth factor, related protein 3	---	cell proliferation	growth factor activity
213428_s_at	25.06	COL6A1	collagen, type VI, alpha 1	---	phosphate transport /// cell adhesion /// cell adhesion	structural molecule activity /// protein binding
206116_s_at	23.28	TPM1	tropomyosin 1 (alpha)	Striated_muscle_contraction	cell motility /// regulation of muscle contraction /// regulation of heart contraction	actin binding /// structural constituent of cytoskeleton /// structural constituent of muscle
236266_at	22.24	LOC283666	Hypothetical LOC283666	---	---	---
231897_at	21.94	LTB4DH	leukotriene B4 12-hydroxydehydrogenase	---	leukotriene metabolic process /// metabolic process	catalytic activity /// alcohol dehydrogenase activity /// binding /// zinc ion binding /// oxidoreductase activity /// 2-alkenal reductase activity /// 15-oxoprostaglandin 13-oxidase activity
208923_at	20.81	CYFIP1	cytoplasmic FMR1 interacting protein 1	---	multicellular organismal development /// nervous system development /// regulation of cell shape /// lamellipodium biogenesis /// lamellipodium biogenesis /// cell differentiation /// ruffle organization and biogenesis /// axon extension	actin binding /// protein binding /// protein binding /// Rac GTPase binding /// actin filament binding
204415_at	14.29	IFI6	interferon, alpha-inducible protein 6	---	release of cytochrome c from mitochondria /// anti-apoptosis /// immune response /// negative regulation of caspase activity /// negative regulation of mitochondrial depolarization	protein binding
206618_at	13.05	IL18R1	interleukin 18 receptor 1	---	immune response /// signal transduction /// signal transduction /// innate immune response	receptor activity /// receptor activity /// transmembrane receptor activity /// interleukin-1 receptor activity
203889_at	10.33	SCG5	secretogranin V (7B2 protein)	---	transport /// intracellular protein transport /// intracellular protein transport /// neuropeptide signaling pathway /// peptide hormone processing /// peptide hormone processing /// regulation of hormone secretion /// regulation of hormone secretion	enzyme inhibitor activity /// enzyme inhibitor activity /// protein binding /// GTP binding /// enzyme activator activity /// unfolded protein binding
225522_at	10.01	AAK1	AP2 associated kinase 1	Fatty_Acid_Synthesis	protein amino acid phosphorylation	nucleotide binding /// protein kinase activity /// protein serine/threonine kinase activity /// ATP binding /// kinase activity /// transferase activity
223276_at	8.6	MST150	MSTP150	---	---	---
224030_s_at	4.149	LOC100130415	similar to PRO2751	---	---	---
241637_at	3.681	---	Transcribed locus	---	---	---
1555448_at	1.972	C14orf108	chromosome 14 open reading frame 108	---	protein complex assembly /// intracellular protein transport /// vesicle-mediated transport	protein binding /// protein transporter activity